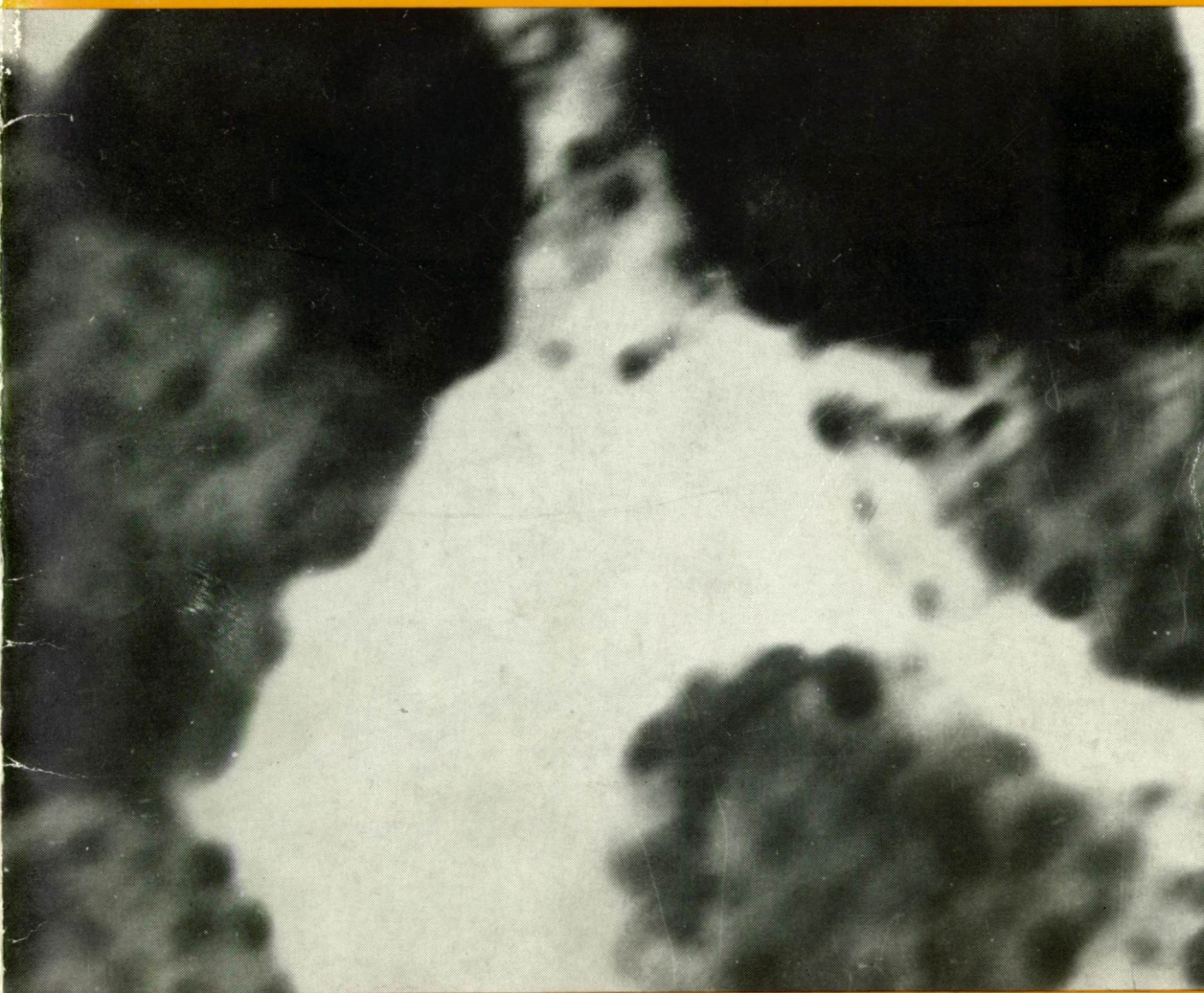


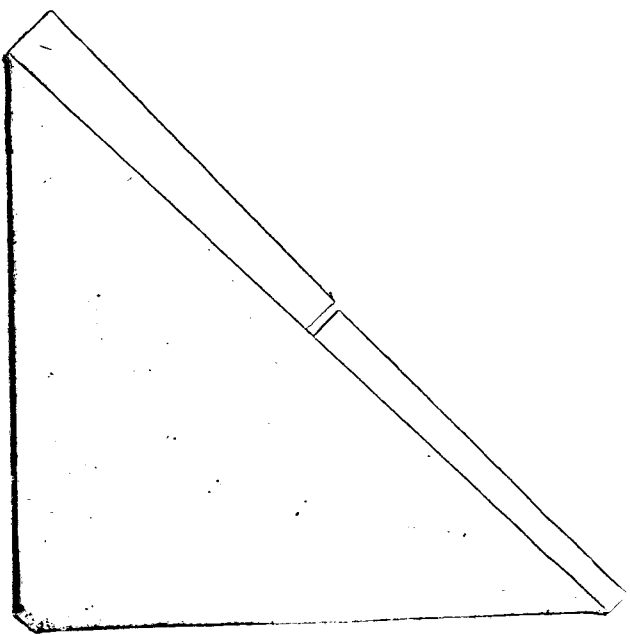
JATE
EGYETEM GYÜJTEMÉNY
HELYBEN OLVASHATÓ!

**PLANT CELL BIOLOGY AND
DEVELOPMENT**
EDITED BY
M. KEDVES



JATE
EGYETEMI
GYUJTEMENY

HELYBEJUTOLVASHATO!



B 135180

Plant Cell Biology and Development

1

Editor: M. Kedves

Financial support by the Grant OTKA—2, 24/88

and by the Foundation for Szeged

SZTE Klebelsberg Könyvtár
Egyetemi Gyűjtemény
2.

**HELYBEN
OLVASHATÓ**



B 135180

ISSN 0866—5443

**1991
Szeged**

Contents

Preface	5
1. High temperature effect on the spores of <i>Equisetum arvense</i> L. KEDVES, M., TÓTH, A. and FARKAS, E.	8
2. First observations on the biopolymer organization of the intine KEDVES, M.	15
3. Biopolymer organization of the partially degraded oil shale with the fragmentation method KEDVES, M. ROJK, I. and VÉR, A.	28
4. Biopolymer organization of partially degraded exines of saccate gymnosperm pollen grains KEDVES, M. PÁRDUTZ, Á. and VÉR, A.	32
5. Basic establishments of the biological objects molecular structure containing quasi — crystalloid skeleton KEDVES, M., PÁRDUTZ, Á., FARKAS, E. and VÉR, A.	35
Chronicle	38

Contributors

Eszter FARKAS

Cell Biological and Evolutionary Micropaleontological Laboratory. University Student.

Miklós KEDVES

Cell Biological and Evolutionary Micropaleontological Laboratory, Research Councilor, Honorary Professor.

Árpád PÁRDUTZ

Institute of Biophysics, Biological Research Center of the Hungarian Academy of Science, Research Ass. Professor.

Imre ROJIK

Faculty of Sciences of the J. A. University, Laboratory of Electron-microscopy. Research Ass. Professor.

Anita TÓTH

Cell Biological and Evolutionary Micropaleontological Laboratory. University Student.

Annamária VÉR

Cell Biological and Evolutionary Micropaleontological Laboratory. University Student.

Preface

Prof. Dr. B. CSÁKÁNY, The Rector of the University J. A. has agreed with the establishment of the Cell Biological and Evolutionary Micropaleontological Laboratory in the Department of Botany in his official communication of 25B—1990 Sz. O. dated on 21st August 1990. The basic purpose of this laboratory is the contribution to multidisciplinary research programs with the collaboration of several researchers or students from foreign countries or institutions. Previously a remarkable number of research programs were completed and their results were published. This place lets me mention the names of colleagues and students as scientific collaborators during the last five years: N. ABOUL ELA (Department of Geology, Cairo University, Cairo, Egypt), E. AMBRUS (Student, Department of Biology, Gyula Juhász High School, Szeged, Hungary), J. CIVIS (Departamento de Paleontologia, Facultad de Ciencias, Universidad de Salamanca, Salamanca, Spain), E. FEJES (Student, Department of Biology, Gyula Juhász High School, Szeged, Hungary) W. M. FELDER (Rijks Geologische Dienst Karteedistrict Zuid, Kantoor Heerlen, AJ Heerlen, The Netherlands), G. GÉVAY (Educational Technology Center, J. A. University, Szeged, Hungary), B. GYEBROVSZKI (Student, Department of Biology, Gyula Juhász High School, Szeged, Hungary), G. F. W. HERNGREEN (Geological Survey of The Netherlands, AD Haarlem, The Netherlands), M. HETÉNYI (Department of Mineralogy, Geochemistry and Petrology, J. A. University, Szeged, Hungary), I. KINCSEK (Department of Biology, Gyula Juhász High School, Szeged, Hungary), L. KÖRMÖCZI (Department of Botany, J. A. University, Szeged, Hungary), J. P. M. T. MEESSEN (Geologisch Bureau, Geological Survey of The Netherlands, AC Heerlen, The Netherlands), E. NAGY (Hungarian Geological Institute, Budapest, Hungary), I. ROJIK (Faculty of Sciences, Laboratory of Electron-microscopy, Szeged, Hungary), J. DE PORTA (Departamento de Paleontologia, Facultad de Geologia, Universidad de Barcelona, Barcelona, Spain), N. SOLÉ DE PORTA (Departamento de Paleontologia, Facultad de Geologia, Universidad de Barcelona, Barcelona, Spain), T. SZEDERKÉNYI (Department of Mineralogy, Geochemistry and Petrology, J. A. University, Szeged, Hungary), J. WINTER (E. M. A. University, Department of Geology, Greifswald, Germany). The purpose, the subjects and the methods of these investigations are heterogeneous, these may be classified in several fields of science. E. g.: Botany, Geology, Geochemistry, Biostratigraphy, Cell Biology, Molecular structure, etc. (This multidisciplinary character is the basis in getting new results by the way of combination of the methods and concepts of different independent laboratories.)

Regarding the teaching program of our laboratory, two different fields may be pointed out:

1. Undergraduate and postgraduate teaching for Hungarian and foreign students and young colleagues. Special lectures from the subjects as follows:

- Basic Palynology, Micropaleontology.
- Biopolymer organization and structural levels of the plant cell wall.
- Quasi-crystalloid modelling of the biopolymer structure of the plant cell wall.
- Different problems of the general Evolution Theory, such as the Supernova Theory and so on.

2. As an attempt, a search for young talented persons for scientific laboratory work was started about three years ago. The best voluntary grammar-school students had the opportunity to work in our laboratory. Thanks for the help in the organization and cooperation of J. BÁNFALVY, M. BOGÁTHY-EKE, and M. JURAY. Several young (teen-ager) scholars learned the elementary disciplines of laboratory work and scientific search. The best grammar-school students are co-authors of several publications on the understanding their activity in the elaboration of the details of researches. Some of the above mentioned papers are under publication. Co-authors as former grammar-school students are as follows: P. AILER, A. BELLON, E. FARKAS, Á. SCHMÉL and A. TÓTH.

E. FARKAS started to work in our laboratory three years ago and now as a university student she continues her work at the same place. It is hoped, that the best of these young students are going to become well-known scientists in the future.

This started as an attempt and now it may be declared this is succesful and also will continue in the future. As regards the education of the youngest students the help of the laboratory assistant I. BIRÓ—HALÁSZ is also emphasize. This series of publication will assure place for the youngest students, too.

Szeged, 7. January, 1991.

M. KEDVES
head of the laboratory

Appendix

List of publications which appeared in cooperation in the last five years

- ABOUL ELA, N. M. and KEDVES, M. (1988): Palynological studies on intercalated sediments of the Yemen volcanics near Sana'a. — Ann. Univ. Sci. Budapestinensis de R.E. nom., Sect. Geol. 28, 27—41.
- GÉVAY, G. and KEDVES, M. (1989): A structural model of the sporopollenin based on dodecahedrane units. — Acta Biol. Szeged 35, 53—57.
- HERNGREEN, G. F. W., FELDER, W. M. KEDVES, M. and MEESSEN, J. P. M. T. (1986): Micropaleontology of the Maestrichtian in borehole Bunde, The Netherlands. — Rev. Palaeobot. Palynol. 48, 1—70.
- HETÉNYI, M. and KEDVES, M. (1986): Organic Geochemical characterization of brown coals by thermal degradation and modified Rock—Eval method. — Acta Miner.—Petr. Szeged 28, 95—108.
- HETÉNYI, M. and KEDVES, M. (1990): Relations between the hydrocarbon genetic features of kerogens and their biological precursor material. — Int. Symp. on Geochem. Prosp., Extended Abstracts, 246.
- KEDVES, M. and KINCSEK, I. (1989a): Quasi-crystalloid biopolymer organization of the fossil spore and pollen wall. — II. European Palaeobot. Conf., Madrid, Abstracts, 16.
- KEDVES, M. and KINCSEK, I. (1989b): Effect of the high temperature on the morphological characteristic features of the sporomorphs I. — Acta Biol. Szeged 35, 233—235.
- KEDVES, M. J., KINCSEK I., AMBRUS, E. FEJES, A. y GYEBROVSZKI, B. (1988): La estructura molecular de la exina en algunos granos de polen bialados de gimnospermas. — VII Simposio de Palinologia, A. P. L. E., Granada, Abstracts, 67.
- KEDVES, M. et KÖRMÖCZI, L. (1985): Sur les problèmes de conservation des sporomorphes dans des conditions differentes. — An. Asoc. Palinol. Leng. Esp. 2, 263—271.
- KEDVES, M. and ROJK, I. (1989): Investigation of the biopolymer organization of partially degraded exines with the fragmentation method. — Acta Biol. Szeged 35, 71—80.
- KEDVES, M. SOLE DE PORTA, N., PORTA DE, J. y CIVIS, J. (1985): Estudio palinologico de los sedimentos Maastrichtienses del Barranco del La Posa (Prepirineo, Lerida, España). — An. Asoc. Palinol. Leng. Esp. 2, 247—253.
- KEDVES, M. and SZEDERKÉNYI, T. (1985): The importance of the spore-pollen investigations in the recognition of the radioactive element content of the lake mud. — Acta Biol. Szeged 31, 215—216.
- KEDVES, M. and SZEDERKÉNYI, T. (1986): Investigation on the microscopic plant remnants and the radioactive element contents of some mud samples of the Hungarian Plain. — Acta Biol. Szeged 32, 209—211.
- KEDVES, M. and SZEDERKÉNYI, T. (1988): Transmission electron microscopical investigation of xylem remains transporting radioactive elements in the mud of Lake Vadkert. — Acta Biol. Szeged 34, 71—81.
- KEDVES, M. and WINTER, J. (1988): Higher organized sporoderm biopolymer units of *Equisetum arvense* L. — Acta Bot. Hung. 34, 361—374.
- NAGY, E. and KEDVES M. (1988): State of Palynological research in Hungary. — Acta Bot. Hung. 34, 311—324.
- PORTA, DE J. KEDVES M., SOLÉ DE PORTA, N. and CIVIS, J. (1985): Palinologia del Maastrichtiense del Barranco de la Posa (Lérida, España). Problemática regional. — Rev. Inv. Geol. 40, 5—28.

1. HIGH TEMPERATURE EFFECTS ON THE SPORES OF *EQUISETUM ARVENSE* L.

M. KEDVES, A. TÓTH and E. FARKAS

Cell Biological and Evolutionary Micropaleontological Laboratory of the Department of Botany of the J.A. University, H—6701, P. O. Box 657, Szeged, Hungary.

Abstract

Freshly collected spores of *Equisetum arvense* L. were examined by light microscopy after different exposures time of high temperature at 200 °C. Qualitative changes observed as follows: Elaters partially or completely separate from the spores. Perispore also folds out from the exospore. These changes and the alterations in maximum spore size are represented in diagrams. No difference is found between the quantitative changes at 200 °C with time, even after 300 hours.

Key words: Palynology, *Equisetum*, high temperature effect.

Introduction

During previous experimental investigations on the thermal alterations at high temperature of Recent pollen grains (KEDVES and KINCSEK, 1989, KEDVES et al., in press), we noted important changes in morphology, which reflect on taxonomy and phylogeny. Early results of similar experiments on spores, especially on the genus *Selaginella* (KEDVES, 1990), did not show equally important changes as a result of high temperature. Our research programme in this field includes all the most important groups of spores and pollen grains. Different concepts are involved, e.g. methods, taxonomy, phylogeny. As the present state of knowledge several problems of method are to be solved. The purpose of the present paper is partly to focus on methods and partly to study the peculiarities of the *Equisetineae* in every respect. Morphological characteristics of the spores of *Equisetum* are dealt with in a previous paper (KEDVES, 1979). TEM data on the spore wall of the genus *Equisetum* were published by GULLVAG (1968), LUGARDON (1969), SAXENA (1980), and SEM data by KEDVES (1979). The biopolymer organisation of the sporoderm of *Equisetum* was studied by KEDVES and WINTER (1988).

Materials and Methods

The investigated material was collected by the senior author on 1. 4. 1989. Locality: left bank of the Tisza River. The spores were frozen at -20°C after collection. For the experiments on high temperature effects 5 mg of spore material were used. Experiments were made as follows:

Number allotted to experiment	length of time	date
645	10'	1. 6. 1989
646	20'	1. 6. 1989
647	30'	1. 6. 1989
648	40'	1. 6. 1989
649	50'	1. 6. 1989
578	1 ^h	3. 4. 1989
579	2 ^h	3. 4. 1989
580	3 ^h	3. 4. 1989
581	4 ^h	3. 4. 1989
582	5 ^h	3. 4. 1989
583	10 ^h	8. 5. 1989
624	25 ^h	8—9. 5. 1989
625	50 ^h	8—10. 5. 1989
638	75 ^h	1—4. 6. 1989
639	100 ^h	1—5. 6. 1989
640	125 ^h	1—6—6. 1989
650	150 ^h	12—18. 6. 1989
761	200 ^h	10—18. 12. 1989
762	250 ^h	10—20. 12. 1989
763	300 ^h	10—22. 12. 1989

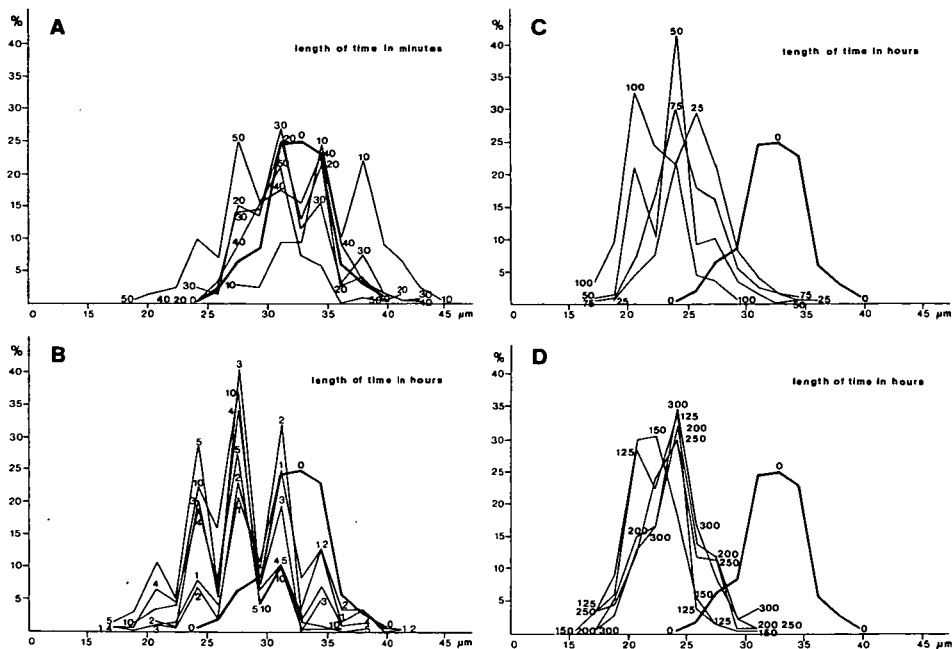
The slides for light microscopy were mounted in glycerin-jelly hydrated at 39,6%. 200 Specimens of each sample were investigated according to the following points of view: 1. Spore diameter. 2. Degree of degradation of the different wall layers, including elaters. 3. Thermal Alteration Index (TAI) in accordance with UTTING et al. (1989).

Results

1. Alterations in the diameter of spores (Text-fig. 1.1., A): After 10 min. at 200°C the spore diameter increased, and two maxima appeared in the frequency distribution diagram. After 20 min. the spore diameter started to decrease. Indeed, after 20 min. heating the frequency distribution graph is nearly the same as that for fresh spores without heating. It is worth mentioning that the frequency distribution of the spores heated during 50 min. is the inverted graph of those heated for 10 min. The degradation of the different sporoderm layers is extremely peculiar (Text-fig. 1. 2., A). During these experiments an unexpectedly regular change has been registered. The highest quantity of complete sporoderms, i.e. exospore + perine + elaters, was observed in spores heated for 50 min. This quantity is higher than that found in spores that were not experimented upon. However, the frequency distribution of spores without elaters changes regularly in relation to the length of time, of heating. Perispore loss was not observed in these experiments.

2. Experiments during 1^{hr} to 10^{hrs} resulted in several maxima in the frequency distribution of the spore diameter (Text-fig. 1. 1., B). The decrease in spore size is more or less regular in relation to the duration of heating. In contrast (Text-fig. 1.



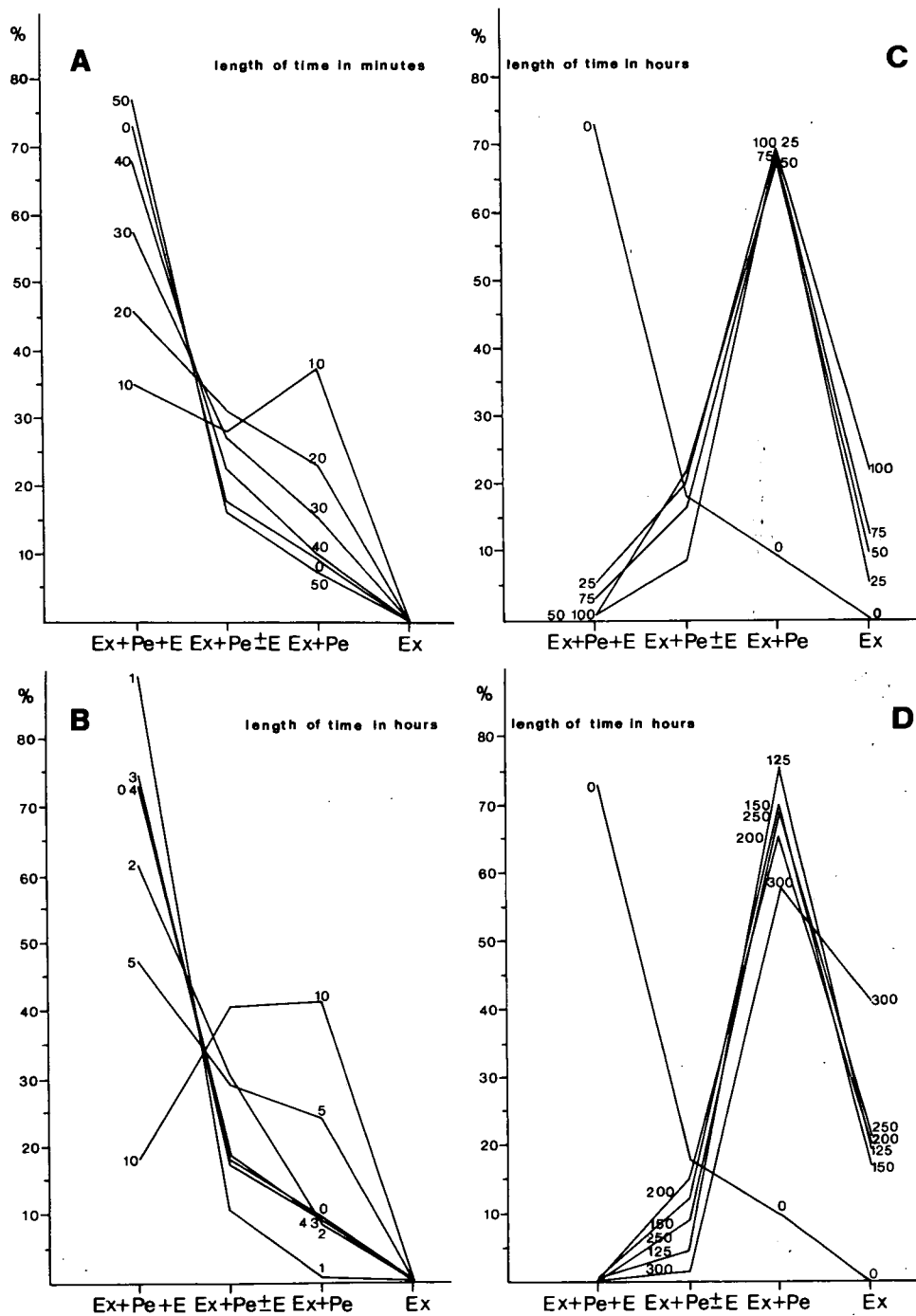


Text-fig. 1.1.
Variation-statistical diagrams of the spores investigated.

1., A), the frequency distribution graphs of spores subjected to experiments are quite different to those of fresh ones. The disappearance of the different sporoderm layers (Text-fig. 1.2., B) develops similarly (Text-fig. 1.2., A). Complete sporoderms were observed after heating for 1^{hr}. Heating during 3^{hrs} and 4^{hrs} brought nearly the same results as before heating. However, peculiar results were obtained after 2^{hrs}, and there the degradation of the sporoderm after heating for 5^{hrs}, and 10^{hrs} is remarkable. The frequency distribution graph of the latter is similar to that for 10^{min}.

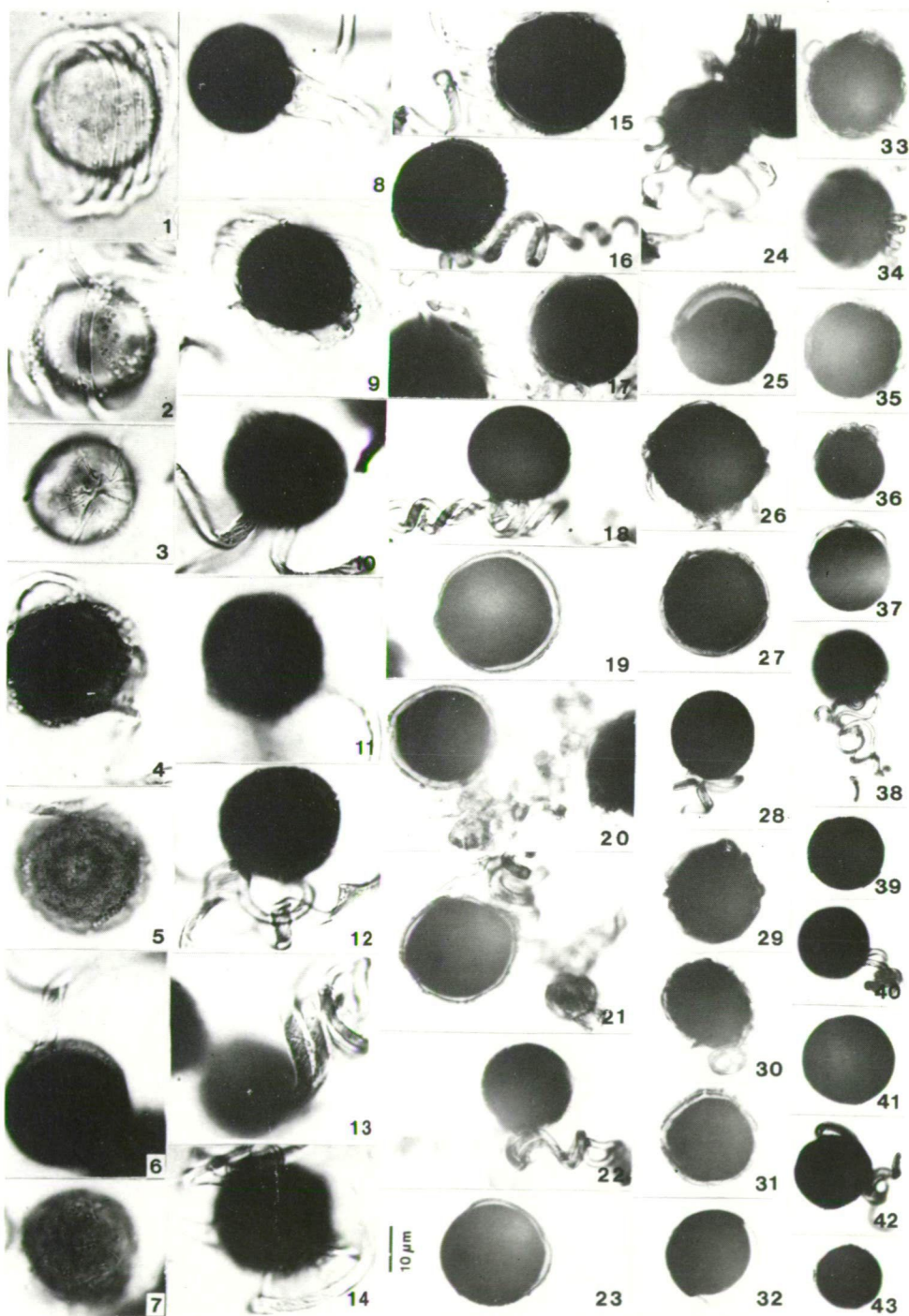
3. Heating the spores for 25^{hrs}, 50^{hrs}, 75^{hrs}, and 100^{hrs}, resulted in a more or less regular decrease in diameter. After 75^{hrs} the frequency distribution graph has two maxima, one of which corresponds to that of spores heated for 50^{hrs}. Changes in sporoderm degradation show about the same tendency. Very few spores bear elaters whereas most conserve perine. In these experiments the spores without perine appeared as a new form of degradation. The quantity of these spores changes regularly in accordance with the length of time of heating.

4. High temperature for 125^{hrs}, 150^{hrs}, 200^{hrs}, 250^{hrs}, and 300^{hrs}, did not result in a notable decrease in spore diameter. Regarding the detail, the two maxima in the frequency distribution graph at 125^{hrs}, and the two flat, more or less identical maxima at 150^{hrs} may be noted. In these experiments, the spores have practically all lost their elaters. However, the percentage of spores with perine (Text-fig. 1. 2., D)



Text-fig. 1.2.

Frequency distribution graph of the degradation process of the different sporoderm layers.
E = elaters, Pe = perispore, Ex = exospore.



◀ Plate 1. 1.

1—43. *Equisetum arvense* L., Recent.

1—3. Spores without staning or heating.

4, 5. Experiment No 645, length of time 10 min.

6, 7. Experiment No 646, length of time 20 min.

8, 9. Experiment No 647, length of time 30 min.

10, 11. Experiment No 648, length of time 40 min.

12, 13. Experiment No 649, length of time 50 min.

14, 15. Experiment No 578, length of time 1 hr.

16, 17. Experiment No 579, length of time 2 hrs.

18, 19. Experiment No 580, length of time 3 hrs.

20, 21. Experiment No 581, length of time 4 hrs.

22, 23. Experiment No 582, length of time 5 hrs.

24, 25. Experiment No 623, length of time 10 hrs.

26, 27. Experiment No 624, length of time 25 hrs.

28, 29. Experiment No 625, length of time 50 hrs.

30, 31. Experiment No 638, length of time 75 hrs.

32, 33. Experiment No 639, length of time 100 hrs.

34, 35. Experiment No 640, length of time 125 hrs.

36, 37. Experiment No 650, length of time 150 hrs.

38, 39. Experiment No 761, length of time 200 hrs.

40, 41. Experiment No 762, length of time 250 hrs.

42, 43. Experiment No 763, length of time 300 hrs.

is nearly the same as previously (Text-fig. 1. 2., C), with the exception of spores heated for 300^{hrs}. Percentual changes in spores without perine are more or less regular except for spores heated during 300^{hrs}.

“Thermal Alteration Index” values are as follows. N. B. — S = spore wall, exospore and perine, E = elaters.

0 (S = 1, E = 1), 10' (S = 1+, E = 1), 20' (S = 2, E = 1+), 30' (S = 2, E = 2—), 40' (S = 2, E = 2—), 50' (S = 2+, E = 2), 1^h (S = 1+, E = 1), 2^h (S = 2, E = 1), 3^h (S = 2+, E = 2), 4^h (S = 2+, E = 2), 5^h (S = 3—, E = 2+), 10^h (S = 3—, E = 2+), 25^h (S = 3—, E = 3—), 50^h (S = 3—, E = 3—), 75^h (S = 3, E = 3), 100^h (S = 3, E = 3), 125^h (S = 3, E = 3), 150^h (S = 4—, E = 3), 200^h (S = 4—, E = 3), 250^h (S = 4—, E = 3), 300^h (S = 4—, E = 4—).

These data refer to the following: the colour of the spores changes gradually corresponding to the length of time of heating. As departures from gradual changes one notes the results after 50^{min}, 1^{hr}, and 2^{hrs}.

Discussion and Conclusions

New results are as follows.

1. Taking into consideration the irregularities in the results of the different experiments the following can be presumed: The diagenesis of the chemistry of the spore-pollen wall was not completely interrupted by freezing at -20°C , and the experiments were not all made at the same time. Another thing is there may also have been differences in the maturity of spore samples in spite of the careful collection of the experimental material.

2. In a previous paper (KEDVES et al., 1990), dealing with inaperturate gymnosperm pollen grains the separation of non-experimental and experimental frequency distribution graphs has been noted. With *Taxus baccata* this is between 50–100 hrs with *Juniperus virginiana* at 125–150 hrs. As was pointed out previously with spores of *Equisetum arvense* heated for 300 hrs this has not happened. Probably, this phenomenon is a consequence of the relatively thick wall of *Equisetum* spores. Taking into consideration the tendencies of the frequency distribution graphs it may be assumed that after a certain diameter/wall thickness ratio the separation of the non-experimental and experimental frequency distribution graphs occurs. This problem needs further investigation.

3. The two maxima occurring occasionally in the frequency distribution of the spores of *Equisetum arvense* after some experiments probably relate to the “sexual dimorphism” of the homosporous spores of this genus, cf. PIÉRART (1974).

4. Spores without elaters or without perispore do occur in fossil material. In this way the altered *Equisetum* spore can be similar and/or identical with some algal cysts.

Acknowledgements

This work was supported by grant OTKA—2, 24/88. The writers are indebted to Dr. R. ZÁNTHÓ for corrections of the English.

References

- GULLVAG, B. M. (1968): On the fine structure of spores of *Equisetum fluviale* var. *verticillatum* studied in the quiescent, germinated and non-viable state. — Grana Palynol. 8, 23–69.
- KEDVES, M. (1979): Testing of the spores in the *Equisetum* genus. — Bot. Közlem. 66, 195–203.
- KEDVES, M. (1990): Experimental investigations on recent *Selaginella* spores. — Taiwania 35, 587–599.
- KEDVES, M. and KINCSEK, I. (1989): Effect of the high temperature on the morphological characteristic features of the sporomorphs I. — Acta Biol. Szeged. 35, 233–235.
- KEDVES, M. and WINTER, J. (1988): Higher organized biopolymer units of *Equisetum arvense* L. — Acta Bot. Hung. 34, 361–374.
- KEDVES, M., TÓTH, A. and FARKAS, E. (in press): Effects of the high temperature on the morphological characteristic features of the sporomorphs. II. — Acta Biol. Szeged.
- LUGARDON, B. (1969): Sur la structure fine des parois sporales d'*Equisetum maximum* LAMK. — Pollen et Spores 11, 449–474.
- PIÉRART, P. (1974): Note préliminaire sur la mesure de spores dispersées fossiles. — Bull. Inst. r. Sci. nat-Belg. 49, 1–17.
- SAXENA, D. K. (1980): Ultrastructure of spore of *Equisetum ramosissimum* and *E. diffusum*. — 5. Int. Palynol. Conf. Abstr. 355.
- UTTING, J., GOODARZI, F. DOUGHERTY, B. J. and HENDERSON, C. M. (1989): Thermal maturity of Carboniferous and Permian rocks of the Sverdrup Basin, Canadian Arctic Archipelago. — Geol. Surv. Canada, Paper 89–19, 1–20.

2. FIRST OBSERVATIONS ON THE BIOPOLYMER ORGANIZATION OF THE INTINE

M. KEDVES

Cell Biological and Evolutionary Micropaleontological Laboratory of the Department of Botany of the J. A. University, H-6701, P.O. Box 657, Szeged Hungary.

Abstract

During our experimental investigations on the recent and fossil sporomorphs characteristic biopolymer units were observed in the intine of partially degraded pollen grains of *Encephalartos ferox* BERTOL. The modified Markham rotation method was used to get information on the symmetry of the basic biopolymer units in Angstrom dimension. A hexagonal basic biopolymer unit was established. The five and/or ten-fold symmetry rotation of the hexagonal unit resulted in not pentagonal primary and secondary symmetry points against the biopolymer units of the ectexine. The quasi-crystalloid basic biopolymer units after 3-, 4-, 6-, 7-, 8-, and 9-fold rotation resulted in well defined secondary points of symmetry. This phenomenon also has been verified peculiar characteristic feature of the regular five-fold symmetry. In this way the regular pentagonal polygon includes all other kinds of basic symmetries, e.g.: the three-square and the square.

Key words: Palynology, *Gymnospermae*, intine, hexagonal basic biopolymer unit.

Introduction

The two principal layers of the pollen wall (exine, respectively intine) were recognized a long time ago. But most of the researches in Palynology were focused on the exine. Some selected opinions are as follows: MARTENS and WATERKEYN (1961), p. 1390: ..“l'intine — c'est-à-dire la vraie membrane cellulaire — est encore mal connue”. HORVAT (1969), p. 16: ..“l'intine est resté jusqu'ici “le parent pauvre””. LE THOMAS (1981), p. 272: “Although still little studied, the intine is sometimes considered relatively homogeneous in structure.” HESSE (1986), p. 315: ..“the mature sporoderm consists of both exine and intine, the latter has been widely neglected through the years.” As regards the most important characteristic features of the intine, the following may be mentioned. The earlier concepts on the basis of the paper of TOMSOVIC (1960):

I. Intina FRITZSCHE 1837 (the inner layer of the sporoderm which is composed of pectin and cellulose and is soluble by acids and alkalis).

A. Euintina KUPRIYANOVA 1955 = Endintine ELLIOT 1951 (Where the intina is two-layered, there the inner layer consisting of cellulose fibrills is impregnated with a pectinous matter).

B. Exintina FRITZSCHE 1837 (the outer layer of the two-layered intina is stronger than the euintina and is, composed of pectin).

Using the TEM method, ROWLEY (1959) established, p. 14: ..“the intine ultrastructure was composed of a network oriented with the largest axis of the reticulations parallel with the endexine.” SAAD investigated circumstantially the third, intermediate layer of the pollen wall, the medine. In a 1966/67 paper he emphasized that this pectic or callose layer is not soluble in 2-aminoethanol. ROWLEY and ERDTMAN (1967) established that the cellulosic intine form microtubules about 240 Å in diameter near the plasma membrane and generally parallel with it. HORVAT (1969), p. 16: “A cause de sa nature organique, cellulosique (ROWLEY et ERDTMAN, 1967), pectocellulosique (ROLAND, 1967), cellulosique et de composés pectiques, ainsi que d’autres constituants de la paroi cellulaire (SKVARLA et LARSON, 1966), l’intine peut être entièrement détruite par l’acétolyse suivant la méthode de ERDTMAN (1960).” P. 17: “L’intine montre parfois une lamellation faiblement ou délicatement concentrique (SCHWANITZ, 1967) ou une lamellation anastomosée et orientée de la couche interne d’une part, et fibreuse d’orientation quelconque, de la couche externe d’autre part (GULLVAG, 1964).” P. 30: “La réaction phosphatasique est mise en évidence pendant le développement de l’intine.” Interesting establishments were published by SKVARLA and ROWLEY (1970), p. 525: “The wall between the channeled region and the cytoplasm, where we find sporopollenin, is without doubt an intine or part of the intine. It is formed at about the time of microspore mitosis, has a fibrillar texture and stained for polysaccharides, including cellulose.” MASCARENHAS (1975), p. 264: “The intine contains microfibrils of cellulose which are held together by a matrix of pectic material and hemicelluloses. Proteins are also present.” On the basis of a paper by HESLOP-HARRISON (1975) the following may be stressed: p. 277: “The intine forms a continuous layer investing the vegetative cell of the mature pollen grain. Unlike the exine it is unsculptured, although it follows the inner surface topography of the outer layer, and the substances of the two are often interbedded. In gross chemical composition the intine is similar to the primary walls of somatic angiosperm cells, with a microfibrillar cellulose component and matrix material of pectic substances and hemicellulose (SITTE 1953, BROOKS, and SHAW, 1971).” P. 279: “In many earlier descriptions of the intine radially disposed tubules traversing the layer, particularly in the vicinity of the apertures, were reported.” “The intine is characterized by its low density to electrons, fibrillar structure, and location between the plasma membrane and nexine”; ROWLEY and DAHL, 1977, p. 214. NILSSON (1978a, b): intine (pollen) — endospore (*Pteridophyta* spores). THANIKAIMONI (1978), p. 235: “Intine is that part of the pollen wall located between the sporopollenin exine and the cytoplasmatic surface. It is often interbedded with the exine but is not itself composed of sporopollenin. In appearance and composition the intine is comparable with the primary plant cell wall and has been characterized by ROLAND (1971) as an amorphous matrix of pectin with infrequent microfibrils (ROWLEY and SKVARLA, 1974)”. COUSIN (1979), p. 124: “The intine is thick and perforated with numerous polysaccharides containing cytoplasmatic channels in the interapertural areas where the exine is thick and granular.” THANIKAIMONI and ROLAND-HEYDACKER (1979), p. 542: “..intine (inner unit of the sporoderm: essentially a mixture of a pectic amorphous matrix and infrequent microfibrils, cf. ROLAND, 1971).” HESLOP-HARRISON, Y. and HESLOP-HARRISON, J. (1982), p. 831: „The early work of SITTE

(1953) showed that the inner wall of the pollen grain, the intine, possesses a microfibrillar component. Since SITTE's observation it has been widely accepted that the microfibrils are cellulosic (see review by LINSKENS, 1967), and thus comparable with those of the primary wall of a somatic cell." SEETHARAM (1985), p. 2: "intine: part of the pollen wall between the exine and the cytoplasm. It lacks sporopollenin and does not resist acetolysis." SOHMA (1985) discussed the nomenclatural problems of the exintine and endintine. HESLOP-HARRISON, Y. et al. (1986), p. 282: "Proteins incorporated in the intine during its deposition are the products of the gametophyte (KNOX and J. HESLOP-HARRISON 1970), but PACINI et al. (1981) have shown that proteins of sporophytic origin may also accumulate at, or actually within, the apertures in many angiosperm pollen." SKVARLA and ROWLEY (1986), p. 397: "The intine is characterized by many narrow and long microvillae-like vaginations which make contact with a manifold-like layer at the base of the channelled zone. Dictyosomes are numerous in the peripheral cytoplasm at this time. Late in development the intine becomes distinctly fibrillar." In resumé the intine is chemically complex: cellulose, hemicellulose, pectin, proteins, sporopollenin. The aim of this paper: To publish the first biopolymer structures in angstrom dimension from the intine, and discuss some methodical and other problems.

Materials and Methods

The material of our investigations, the pollen grains of *Encephalartos ferox* BERTOL, was received from the Botanical Garden of Coimbra, Portugal, in 1972. For the first experimental studies air dried material was used, with the aim to investigate the biopolymer organization of the partially degraded exine. Between several experiments No 181 resulted biopolymer structures in the intine, the above mentioned experiment was as follows:

20 mg air dried pollen grains + 1 ml 2-aminoethanol, temperature 30 °C, length of time: 24^h, + 10 ml KMnO₄ aq. dil., temperature 30 °C, length of time 24^h. The washed material was fixed with OsO₄ aq. dil., embedded in Araldite. The ultra-thin section was made in the EM Laboratory of the Biological Centre of the Hungarian Academy of Sciences, on a Porter Blum ultramicrotome. The TEM pictures were taken in the EM Laboratory of the Faculty of Science of the A.J. University, Szeged, on a Tesla BS-500 transmission electron microscope, resolution 6Å. The rotation pictures were made in the Department of Botany of the A.J. University, Szeged.

Results

The experiment moderately degraded the ectexine (Plate 2. 1., fig. 1, 4). Fig. 4 well represents the ultrastructure of the inter-apertural ectexine. The tectum (T), the alveolar infratectal layer (I), and the foot layer (F) are distinctly shown. Near the sulcus (Plate 2. 1., fig. 1), the ectexine was sectioned in tangential plane. Elements of the infratectal layer well shown. The approximatively radially oriented lamellar elements surround in cross-section polygonal spaces. The intine, near the apertural area is well shown, and protruding from the germinal aperture. The granular ultrastructure of the intine is characteristic below the foot layer, but particularly in the protruding apertural intine. The division into two parts of the apertural intine is perceptible only at one part of the apertural intine. In the high magnified pictures characteristic globular biopolymer units were observed (Plate 2. 1., figs. 2, 3, and plate 2.3.). The diameter of these globular elements is 6–7–8 Å.

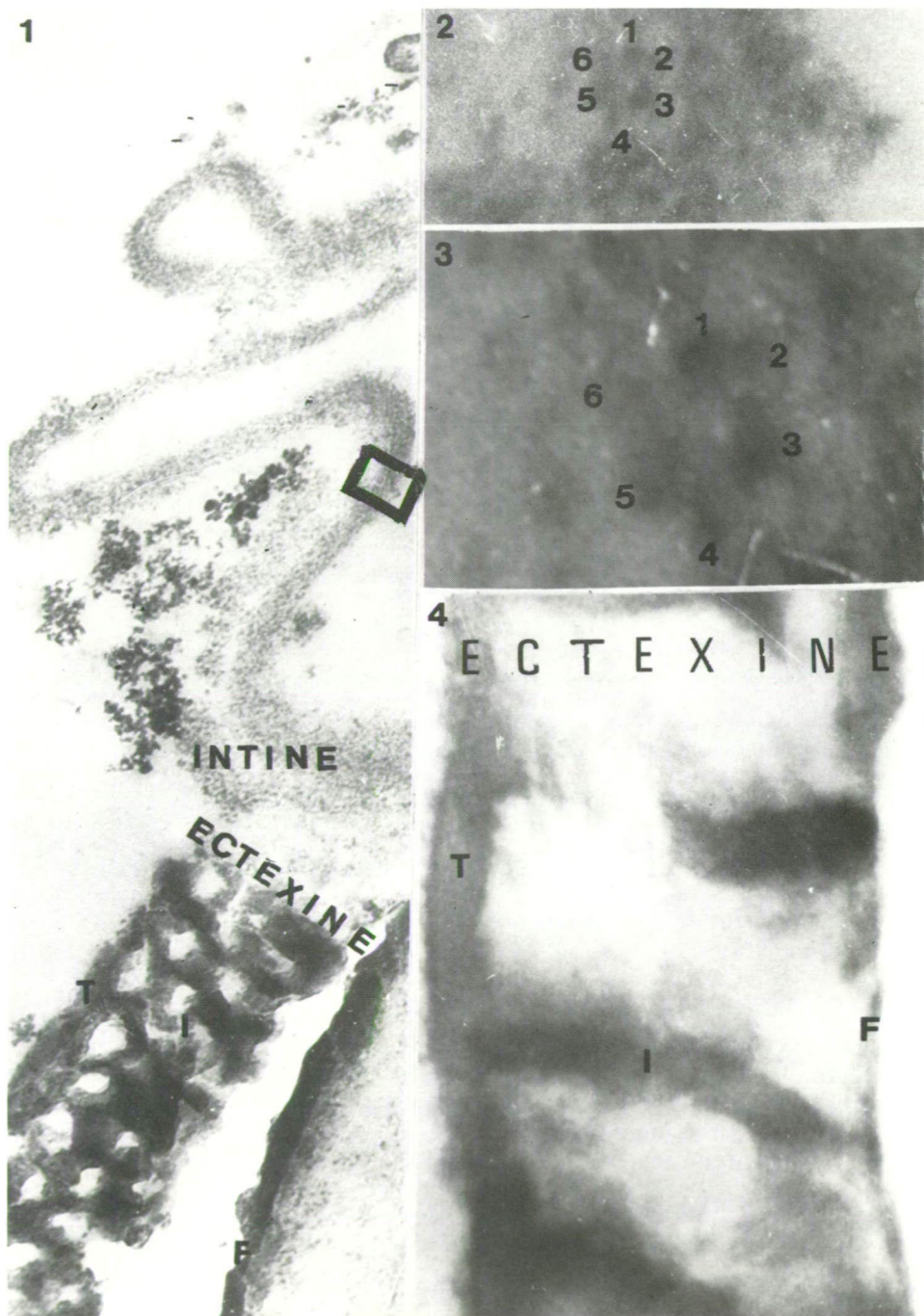


Plate 2.1.

1—4. *Encephalartos ferox* BERTOL. Ultrastructure of the pollen wall after partial degradation with experiment No 181.

1. TEM picture from the apertural area of the pollen grain. Different layers of the inter-apertural ectexine are well shown: tectum (T), alveolar infratectal layer, in this picture in tangential section (I), and the foot layer (F). The germinal intine is protruding. The intine part, which was investigated in the point of view of the biopolymer units is marked with a frame. Negative no: 7964, x50000.

2, 3. Magnified details from the part of the intine investigated in detail. The globular biopolymer units are marked with numbers. Negative no: 7966.

2. x500000.

3. x1250000.

4. Ultrastructure of the inter-apertural ectexine. Negative no: 7977, x200000.

T = tectum, I = infratectum, F = foot layer.

The arrangement of these biopolymer structures into polygonal units was established. In the non-rotated picture of Plate 2. 3., five to six angular biopolymer structures were observed.

To investigate closely the biopolymer organization of the intine, one so-called etalon basic biopolymer unit was chosen. This is situated in the inner part of the intine. This part is framed in fig. 1, in Plate 2.1. This, probably sixangular biopolymer unit is represented in higher magnified pictures in figs. 2 and 3, Plate 2.1.

The modified Markham rotation (cf. KEDVES 1989c), was used in the following mode of action:

Rotation: C.P.6.A.6.6. (Plate 2.2. fig. 1).

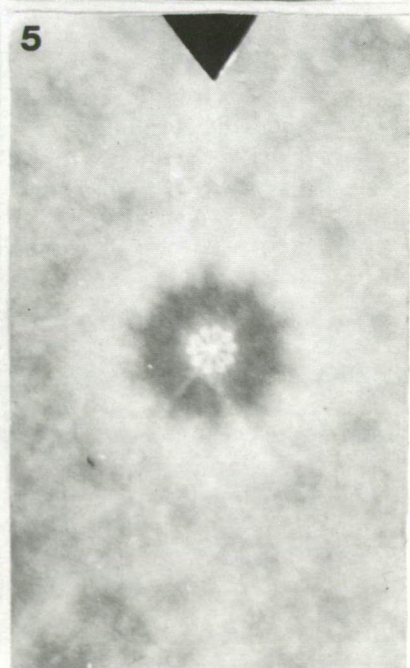
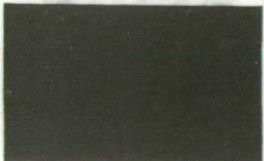
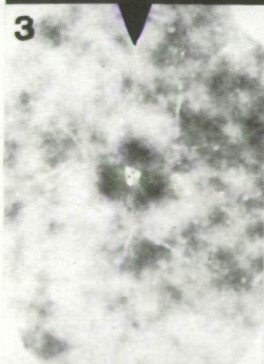
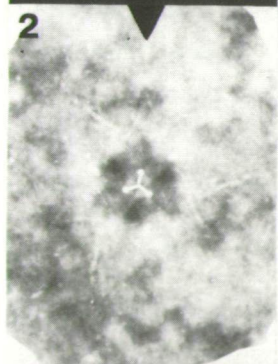
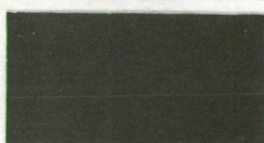
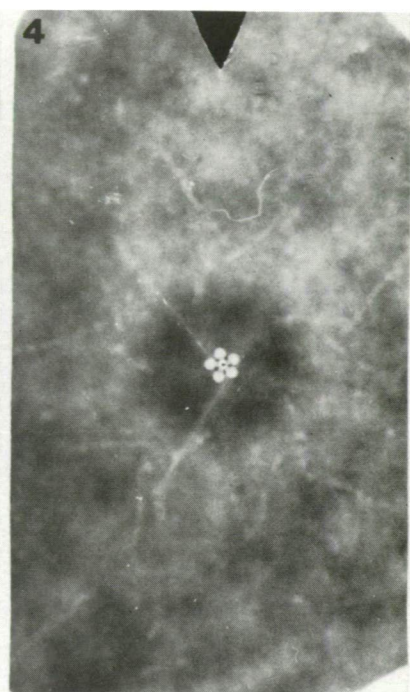
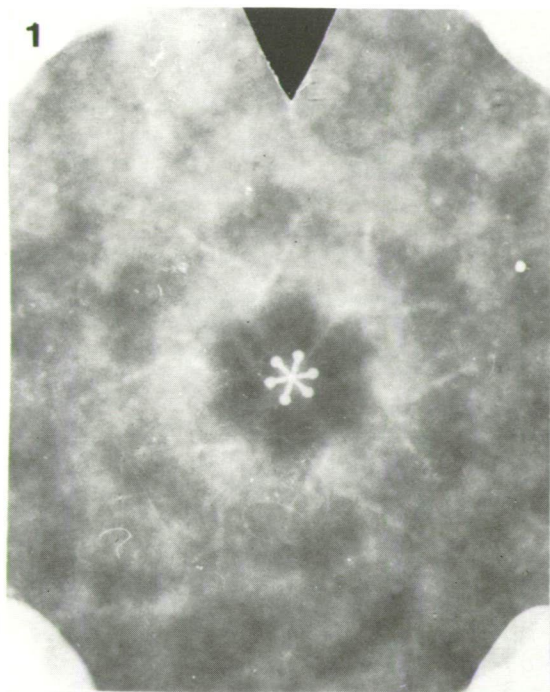
This kind of rotation well reinforced the six globular biopolymer units. These globular elements are much more nearer to one another than at the regular basic pentagonal polygon units of the sporopollenin of the ectexine. Moreover the connectives between the globular elements are not perceptible against the above mentioned biopolymer units of the ectexine. The diameter of the hexangular polygon after rotation: 22 Å. Around the hexangular biopolymer unit, there is a 6—8 Å wide light-coloured zone. This is followed by a darker zone composed from six not so characteristic points of symmetry are not definite.

Rotation: I.P.6.A.6.3.

This kind of rotation may be achieved in different ways. Firstly, the first exposition was made to reinforce the globular biopolymer unit intersected by the P.A. axis. Thereafter the photographic paper was turned in the axis of P and the 3rd globular unit. Finally the last exposition was made in the axis of point P and 5th globular unit. This kind of rotation was designated as follows:

Rotation: I.P.6.A.6.3a (Plate 2.2., fig. 2, text-fig. 2.1.).

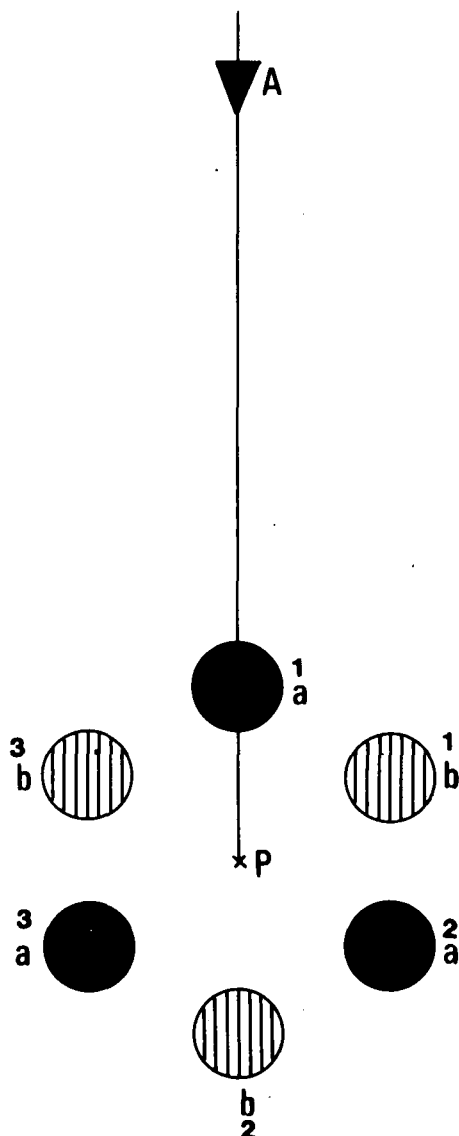
It is interesting that in this case of rotation the 2nd, 4th and 6th globular units were reinforced. Two triangles appeared in cross position. Further zones around the partially rotated biopolymer unit appeared approximatively as at the previously discussed rotation C.P.6.A.6.6.



◀ Plate 2.2.

Encephalartos ferox BERTOL., biopolymer unit of the intine after partial degradation with experiment No 181, and different kinds of rotation.

1. Rotation: C.P.6.A.6.6., x1000000.
2. Rotation: I.P.6.A.6.3a., x500000.
3. Rotation: I.P.6.A.6.3b., x500000.
4. Rotation: C.P.6.A.5.5., x1000000.
5. Rotation: C.P.6.A.5.10., x1000000.



Text-fig. 2.1. Scheme of the incomplete three-fold rotation.

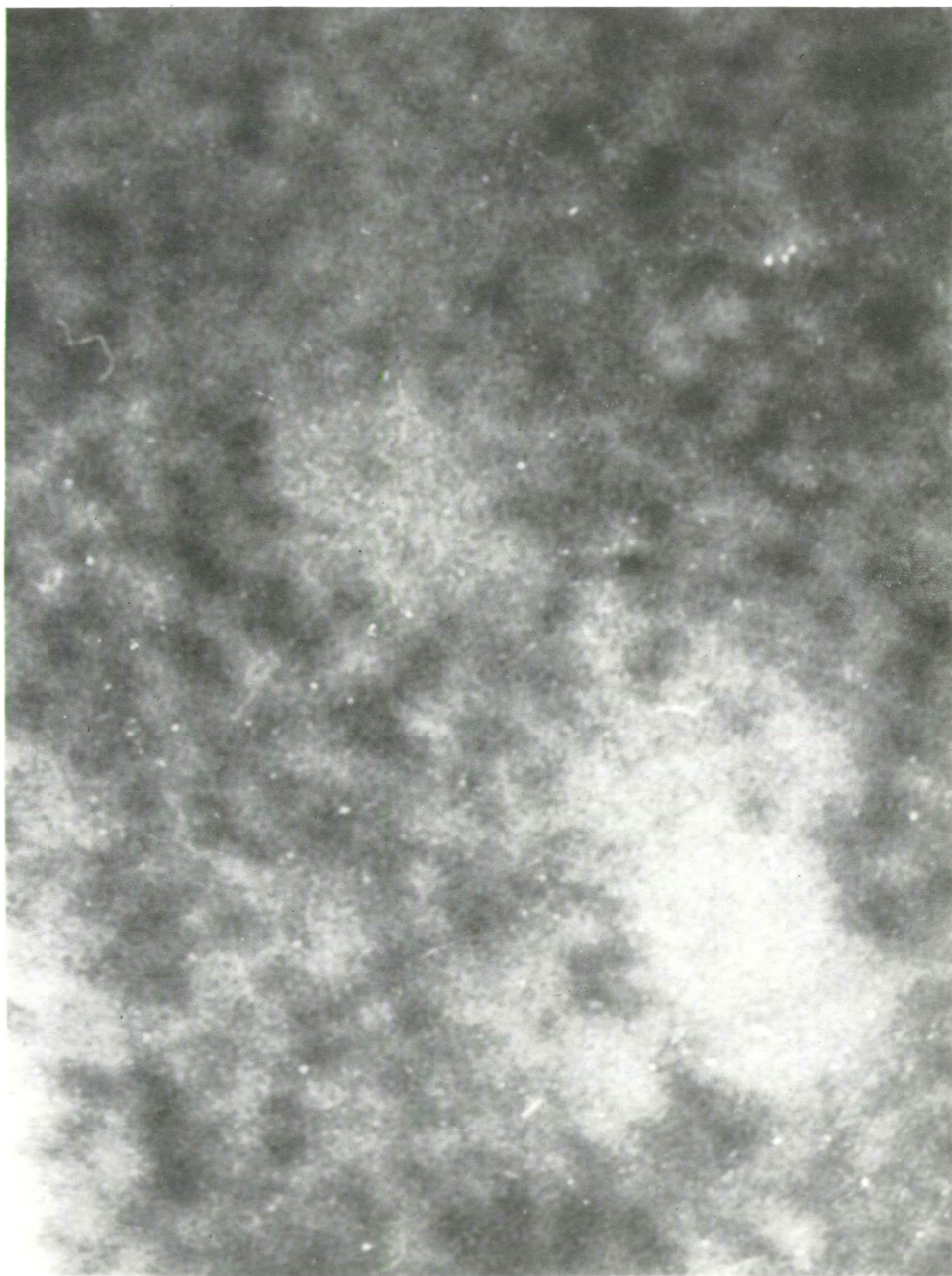


Plate 2.3.

Encephalartos ferox BERTOL., biopolymer units of the intine after partial degradation with experiment No 181. Well shown are the globular biopolymer units and its arrangements in hexagonal and/or pentagonal units. Negative no: 7968, x1250000.

Rotation: I.P.6.A.6.3b (Plate 2.2., fig. 3, text-fig. 2.1.).

This kind of rotation started with the exposition of the 2nd globular biopolymer unit, followed by the 4th and 6th. In this way essentially the 1st, 3rd, and 5th biopolymer units were reinforced, but in this case the reinforced units are not so characteristic as in the previous case.

Methodically it is important to control the symmetry. For this reason, the sexangular biopolymer basic unit was rotated by the five-fold symmetry, too.

Rotation: C.P.5.A.5.5. (Plate 2.2., fig. 4.).

It is well shown that this kind of rotation resulted not a characteristic pentagonal polygon biopolymer unit. An extremely indistinct pentagon appeared, but without characteristic secondary points.

Rotation: C.P.5.A.5.10. (Plate 2.2., fig. 5.)

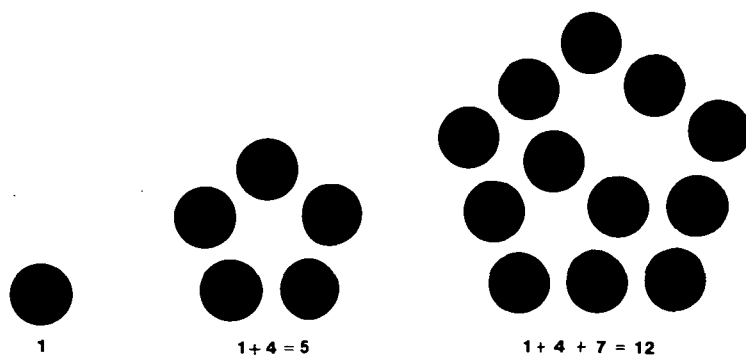
Similarly to the previous rotation this method neither resulted in characteristic points of symmetry.

Discussion

1. As it was emphasized by several authors, cited in the introduction, the researches of the intine were neglected in contrast to the exine. This establishment is valid to the biopolymer organization of this layer. This paper presents the first results in this respect. It seems to be important that the biopolymer unit revealed for the first time is of a hexangular symmetry against the quasi-crystalloid basic biopolymer units of the exine. Naturally, it is necessary to continue further researches in this field. Taking into consideration the up-to-date knowledge of the biopolymer organization of the exine it may be presumed that the intine will also be heterogeneous in this respect. Probably the *Helix* enzyme method will be more successful in the researches of the intine because of its cellulosic content.

2. The Markham rotation method applied to the investigations of the structural organization of the quasi-crystalloid skeleton of the exine was discussed and elaborated in several papers, cf. KEDVES (1989a-d), KEDVES et al. (in print). The basic biopolymer unit, the regular pentagonal polygon in Angstrom dimension needed verification by several ways. Its peculiar characteristics are newly under elaboration. The incomplete rotation and the rotations by 2, 3, 4, 6, 7, 8, and 9-fold symmetry at the basic biopolymer etalon resulted in very characteristic biopolymer points of symmetry. This is also an argument for the peculiar symmetry characteristic of the regular pentagonal polygon as it was established a long time ago. It seems that presence of fivefold symmetry in the structure involves presence of other kinds of rotational symmetries. We are referring here to the old Pythagoreans who gave a polygonal representation of the natural members and showed how the pentagonal numbers do include other ones (cf. text-fig 2, of the book of SAIN 1986; cf. ORE 1977). Of course, our observation still requires closer analysis.

In this way it is not surprising, that the regular pentagonal biopolymer unit has



Text-fig.2.2. Scheme of the Pythagorean pentagon numbers.
After SAIN (1986), modified.

been rotated successfully corresponding to different symmetries. The statements of HEILBRONNER (1986) concerning the symmetry in the Chemistry is important; p. 112: Wie zu erwarten, ist nun die Zahl der zusätzlichen Symmetrieelemente, die man neben den beiden Translationen einbauen kann, größer als im eindimensionalen Fall. Es läßt sich zeigen, daß es auch hier nur eine endliche Zahl von prinzipiell verschiedenen Typen geben kann, die übrigens mit 17 erstaunlich niedrig ist. Einer der Gründe für diese drastische Beschränkung ist, daß die Zahl der Drehungen, welche ein solches zweidimensionales Muster zuläßt, sich auf die Symmetrieeoperationen C_2 , C_3 , C_4 und C_6 beschränkt. Insbesondere ist zum Beispiel C_5 verboten.

3. Finally the prospects and the first results of the biopolymer organization of the plant cell wall, and its aspects:

3.1. New energy basis (KEDVES, 1986, 1987). The quasi-crystalloid biopolymer skeleton of the plant cell wall is extremely unstable. Scanning effect may explode the pollen grains. KEDVES (1987, p. 163): .."it may be hoped that with a rentable technology the oil shale can be a new energy basis, by the liberation of the binding energy of the wall biopolymer structure."

3.2. Coal pulver explosion (KEDVES, 1989a, b). The coal pulver of the explosion dangerous coal mines at Komló were investigated experimentally. Fossil quasi-crystalloid biopolymer structures were demonstrated, and different kinds of the modified Markham rotation were also applied. The taphonomical processes of the sedimentation may discover the quasi-crystalloid skeleton of the plant cell wall, and this extremely labile dry coal pulver for an initial energy may explode. It may also be presumed that the unstable coal pulver may be also an energy basis.

3.3. Modeling of the biopolymer structures resulted in new things. Concerning this subject, the following may be pointed out; KEDVES (1988a): "More highly organized globular structures (KEDVES et al., 1974) and helical structures (ROWLEY, 1980) are derived from the basic polygon elements. These, in turn, are similar to the structure of solar and galactic systems, and also to microbial entities such as

Micrococcus, *Spirillum* and *Treponema*. Viruses are in general crystalloids. An exception is the AIDS virus which is modeled after a pulsar or neutron star cf. Crab Nebula (TUCKER, 1976), MACKAY (1982, p. 517), following KEPLER's concept (1596), stated that "the five regular polyhedra were the 'spherical harmonies' which were the key to the structure of the Solar System". This is mirrored in the biopolymer organization of the sporoderm. Earth expansion (KREMP, 1984) (+) Sun collapsing (—) is part of an equilibrium in the solar system?"

In general it seems that several new ideas published by the K—TEC research program group are very useful for further multidisciplinary researches.

3.4. As a new concept, the cytoskeleton structures and the highly organized biopolymer units may be mentioned. There is an extreme similarity between the two structures. For the cytoskeleton — the gelatinous part of the cytoplasm, — see the review of LISZT and FRIDVALSZKY (1989), for the highly organized biopolymer structures of the sporoderm the paper of the writer (1989d). On the other hand the sporopollenin deposition on unit membranes was established a long time ago, cf. ROWLEY and SOUTHWORTH (1967); synthesis by ABADIE et al. (1986—1987). It may be presumed that in consequence of analogies in nanometer dimension between the cytoskeleton and the highly organized biopolymer units of the sporopollenin, the quasi-crystalloid structure in the cytoskeleton may be present. When the biopolymer structure corresponding to the so-called PENROSE units KEDVES (1989d) may be established in the cytoskeleton, this will be a new aspect to cell biology. *Probably this will bring new ideas to the pathological cell-division, for example to some kinds of cancer.* For the biological quasi-crystalloid structures the new concept of the quasi-crystals is very important namely that entropy stabilizes them. MADDOX (1989), p. 261: .."by different methods to arrive at a convincing demonstration that the stability of real quasicrystalline systems arises not because of some decisive energetic advantages but because of the large entropy of these systems."

If entropy has a certain importance in the biological quasi-crystalloid structures cell biology and cell biophysics will have further perspectives. It is hoped that the results achieved on the biopolymer organization of the spore-pollen wall and other kind of plant cell walls will be useful in further investigations of cell biology.

Acknowledgements

These researches were supported by the grant OTKA—2, 24/88. The writer expresses his thanks to Dr. I BAGI, Dr. I. ROJIK, É. MÁRAMAROSI, and A. TÓTH for their contributions in the elaboration of this problem.

References

- ABADIE, M., HIDEUX, M. and ROWLEY, J. R. (1986—1987): Ultrastructural cytology of the anther. II. Proposal for a model of exine considering a dynamic connection between cytoskeleton, glycolemma and sporopollenin. — *Synthesis*. — *Ann. Sci. Nat. Bot. Paris* 8, 1—16.
- BROOKS, J. and SHAW, G. (1971): Recent development in the chemistry, biochemistry, geochemistry and post-tetrad ontogeny of sporopollenin derived from pollen and spore exines. In *Pollen: development and physiology* (ed. J. HESLOP-HARRISON), 99—114, Butterworth, London.
- COUSIN, M.-TH. (1979): Tapetum and pollen grains of *Vinca rosea* (*Apocynaceae*). — *Grana* 18, 115—128.
- ERDTMAN, G. (1960): The acetolysis method. A revised description. — *Svensk bot. Tidskr.* 54, 561—564.
- GULLVAG, B. (1964): The fine structure of the pollen grain of *Clivia miniata*. — *Grana Palynologica* 5, 253—263.
- HEILBRONNER, E. (1986): Über die Symmetrie in der Chemie. — *Jb. Akad. Wiss. Göttingen*, 78—121.
- HESLOP-HARRISON, J. (1975): The Cronian Lecture, 1974. The physiology of the pollen grain surface. — *Proc. R. Soc. London, B*, 190, 275—299.
- HESLOP-HARRISON, Y. and HESLOP-HARRISON, J. (1982): The Microfibrillar Component of the Pollen Intine: Some Structural Features. — *Abb. Bot.* 50, 831—842.
- HESLOP-HARRISON, Y., HESLOP-HARRISON, J. S. and HESLOP-HARRISON, J. (1986): Germination of *Corylus avellana* L. (Hazel) pollen: Hydration and the function of the oncus. — *Acta Bot. Neerl.* 35, 265—284.
- HESSE, M. (1987): Who do we investigate the intine ultrastructurally? — XIV Int. Bot. Congr. Berlin (West) Germany, Abstracts, 315.
- HORVAT, F. (1969): Localisation en microscopie électronique, de la phosphatase acide dans l'intine de la microspore, chez *Tradescantia paludosa* A. et W. — *Grana Palynologica* 9, 16—33.
- KEDVES, M. (1986): Explosion of pollen grains under the electron beam effect of the scanning electron microscope. — *Acta Biol. Szeged.* 32, 207—208.
- KEDVES, M. (1987): Higher organized sporopollenin biopolymer structures and the explosion of the pollen grains under scanning effect. — *Acta Biol. Szeged.* 33, 163—165.
- KEDVES, M. (1988a): Degrees of biopolymer organization of the sporoderm as a contribution to the new concept of global Geosphere-Biosphere modeling. — 21st Ann. Meet., A.A.S.P., Program and Abstracts, Houston, Texas, USA.
- KEDVES, M. (1988b): Quasi-crystalloid basic molecular structure of the sporoderm. — 7 Internat. Palynol. Congr. Brisbane, Abstracts, 82.
- KEDVES, M. (1988c): About the symmetry of the pentagonal basic biopolymer units of the pollen wall. — *Acta Biol. Szeged.* 34, 157—159.
- KEDVES, M. (1989a): Transmission electron microscopical investigations on partially degraded plant cell walls. — Vth Symposium of the Hungarian Plant Anatomy, Abstracts, 22.
- KEDVES, M. (1989b): New trends in micropaleontological researches. — II European Palaeobotanical Conf., Abstracts, 3.
- KEDVES, M. (1989c): Méthode d'étude des biopolymères de la paroi pollinique à structure quasi-cristalloïde. — *Revue de Micropaléontologie* 32, 226—234.
- KEDVES, M. (1989d): Quasi-crystalloid biopolymer structures of the sporoderm and its highly organized degrees. — *Acta Biol. Szeged.* 35, 59—70.
- KEDVES, M., STANLEY, E.A. and ROJIK, I. (1974): Observations nouvelles sur l'ectexine des pollens fossiles des Angiospermes de l'Éocène inférieure. — *Pollen et Spores* 16, 425—437.
- KEDVES, M., TÓTH, A. FARKAS, E., BELLON, A. and SCHMÉL, Á. (in print): Methodical problems of the biopolymer organization of partially degraded ectexine. — *Ann. Univ. de Rolando Eotvos Nom. Geol.*
- KNOX, R.B. and HESLOP-HARRISON, J. (1970): Pollen-wall proteins: localisation and enzymatic activity. — *J. Cell. Sci.* 6, 1—27.
- KREMP, G.O.W. (1984): The Oldest Traces of Life and the Advancing Organization of the Earth (Part III: Epilogue). — *Paleo Data Banks* 21, 157—396.
- LE THOMAS, A. (1981): Ultrastructural characters of the pollen grains of African *Annonaceae* and their significance for the phylogeny of primitive Angiosperms (first part). — *Pollen et Spores* 22, 267—342.
- LINSKENS, H.F. (1967): Pollen. In: *Handbuch der Pflanzenphysiologie* (ed. H. F. LINSKENS), 18., Springer-Verlag, Berlin and New York.

- LISZT, K. and FRIDVALSZKY, L. (1989): The cytoskeleton and its role in cell development. — Vth Symposium of the Hungarian Plant Anatomy, Abstracts, 27.
- MACKAY, A.L. (1981): De Nive Quinquangula: On the pentagonal snowflake. — Sov. Phys. Crystallogr. 26, 517—522.
- MADDOX, J. (1989): Quasicrystals stabilized by entropy. — Nature 340, 261.
- MARTENS, P. and WATERKEYN, L. (1961): Sur les membranes des pollens à "ballonnets" des Conifères. — Comptes rendus 252, 1390—1393.
- MASCARENHAS, J.P. (1975): The biochemistry of angiosperm pollen development. — Bot. Rev. 41, 259—314.
- NILSSON, S. (1978a): Symposium palynological terminology: Conclusions. — IV Int. Palynol. Conf. Lucknow (1976—77) 1, 189—190.
- NILSSON, S. (1978b): On palynological terminology — Aspects and prospects. — IV Int. Palynol. Conf. Lucknow (1976—77) 1, 218—221.
- ORE, O. (1977): Invitation to Number Theory Random House, New York. Hungarian translation, Gondolat, Budapest.
- PACINI, E., FRANCHI, G. and SARFATTI, G. (1981): On the widespread occurrence of poral sporophytic proteins in pollen of dicotyledons. — Ann. Bot. 47, 405—408.
- ROLAND, F. (1967): Différenciation du sporoderm chez *Ficaria ranunculoides* MOENCH. Observation et évolution des "corps d'Ubisch". — Pollen et Spores 9, 415—425.
- ROLAND, F. (1971): Characterization and extraction of the polysaccharides of the intine and of the generative cell wall in the pollen grains of some *Ranunculaceae*. — Grana 11, 101—106.
- ROWLEY, J.R. (1959): The fine structure of the pollen wall in the *Commelinaceae*. — Grana Palynologica 2, 3—31.
- ROWLEY, J.R. and DAHL, A.O. (1977): Pollen development in *Artemisia vulgaris* with special reference to glycocalyx material (1). — Pollen et Spores 19, 170—284.
- ROWLEY, J.R. DAHL, A.O. and ROWLEY, J.S. (1980): Coiled construction of exinous units in pollen of *Artemisia*. — 38th Ann. Proc. Electron Microscopy Soc. Amer., San Francisco, California, 252—253.
- ROWLEY, J.R. and ERDTMAN, G. (1967): Sporoderm in *Populus* and *Salix*. — Grana Palynologica 7, 517—567.
- ROWLEY, J.R. and SKVÁRLA, J.J. (1974): Origin of the inner intine in pollen of *Canna*. — 32nd Ann. Proc. Electron Microscopy Soc. Amer., St. Louis, Missouri, 2.
- SAAD, S.I. (1966/67): Further evidence in support of the "medine" as a third distinct layer in the pollen wall. — J. of Palynology 2 and 3, 10—16.
- SCHWANITZ, G. (1967): Untersuchungen zur post-meiotischen Mikrosporogenese. I. Morphogenese des *Ruppia*-Pollens. — Pollen et Spores 9, 9—48.
- SEETHARAM, Y.N. (1985): Clusiaceae: Palynology and Systematics. — Inst. Français de Pondichéry, Trav. Sect. Sci. et Techn. 21, 80 pp, 52 pl.
- SITTE, P. (1953): Untersuchungen zur submikroskopischen Morphologie der Pollen- und Sporenmembranen. — Mikroskopie 8, 290—299.
- SKVARLA, J.J. and LARSON, D.A. (1966): Fine structural studies of *Zea mays* pollen. I. Cell membranes and exine ontogeny. — Ann. J. Bot. 62, 1112—1125.
- SKVARLA, J.J. and ROWLEY, J.R. (1970): The pollen wall of *Canna* and its similarity to the germinal apertures of other pollen. — Amer. J. Bot. 57, 519—529.
- SKVARLA, J.J. and ROWLEY, J.R. (1986): *Canna generalis*, the conjectured function of intine-like components. — In: Pollen and Spores: Form and Function, 397—399.
- SOHMA, K. (1985): Ultrastructure of pollen wall of *Lindera umbellata* THUNB. var. *membranacea* (MAXIM.) MONSIYAMA (*Lauraceae*). — Sci. Rep. Tohoku Univ. 4th ser (Biology) 39, 13—19.
- THANIKAIMONI, G. (1978): Pollen morphological terms: Proposed definitions -1-. — IV Int. Palynol. Conf. Lucknow (1976/77) 1, 228—239.
- THANIKAIMONI, G. and ROLAND-HEYDACKER, F. (1979): Pollen morphology of primitive angiosperms: some neglected aspects. — IV. Int. Palynol. Conf. Lucknow (1976/77) 1, 542—545.
- TOMSOVIC, P. (1960): Bemerkungen zum Feinbau des Sporoderms und zu seiner Terminologie. — Preslia 32, 163—173.
- TUCKER, W.H. (1976): The effect of a nearby supernova explosion on the Cretaceous-Tertiary environment. — Syllogeus 12, 111—121.

3. BIOPOLYMER ORGANIZATION OF THE PARTIALLY DEGRADED OIL SHALE WITH THE FRAGMENTATION METHOD

M. KEDVES₁, I. ROJK₂ and A. VÉR₃

1,3. Cell Biological and Evolutionary Micropaleontological Laboratory of the Department of Botany of the J.A. University, H-6701, P.O. Box 657, Szeged Hungary. 2. Faculty of Sciences, Laboratory of Electron-microscopy, P.O. Box 553, Szeged, Hungary.

The plant microfossils of the oil shale were first investigated by WODEHOUSE (1933) from the Eocene of Green River (Colorado, USA). A great number of papers were published later dealing with the occurrence and importance of the *Botryococcus* alga, the major component of the oil shale (e.g.: RAO and MISRA, 1943, TRAVERSE 1955, MAZANCOVÁ, 1960, SAH and KAR, 1970, MARTIN, 1973, MILDENHALL, 1977). TEM data of the fossil *Botryococcus braunii* KÜTZ. from the Upper Tertiary oil shale of Pula (Hungary) were published by KEDVES (1983). BERKALOFF et al. (1983) established that the resistant polymers of the outer wall of *Botryococcus braunii* do not derive from carotenoids. In this way it cannot be considered as sporopollenin (cf. KADOURI et al., 1988). LARGEAU et al. (1986) investigated the immature Torbatine by pyrolysis and the resistant biopolymer (PRB A) was isolated from extant alga (*B. braunii*). KEDVES (1986a) investigated with the LM and TEM the partially degraded colonies of *B. braunii* with the *Helix* enzyme method and with merkapto-ethanol. Globular units arranged into filaments were observed. Another paper (KEDVES, 1986b) summarizes the results of combined investigations of the oil shale of Pula. Using the thin-layer chromatography, pigment remnants were demonstrated.

The first comprehensive model of the biopolymer organization of the sporoderm attempted to synthesize the different molecular levels (KEDVES, 1989). On the basis of the configuration of the basic quasi-crystalloid biopolymer structures the sub-units were modelled in nanometer dimension. Some of these biopolymer structures are represented on the Fig. 1, p. 63. It were not certified at the time of the compilation of this scheme.

Another research program started with the aim to elaborate new methods to get direct information about the biopolymer structures in different levels. The first results of the Recent pollen grains of *Alnus glutinosa* (L.) GAERTN. were remarkable in this point of view (KEDVES and ROJK, 1989). On the TEM picture of P. 75 the basic biopolymer units, are well shown, arranged in spherical ones. These globular units form a network. In this way three kinds of biopolymer organization levels are represented in one TEM picture. We need to emphasize, that not only the advantages, but also the disadvantages were pointed out in the above mentioned paper. On the basis of the first positive results this method were or will used to

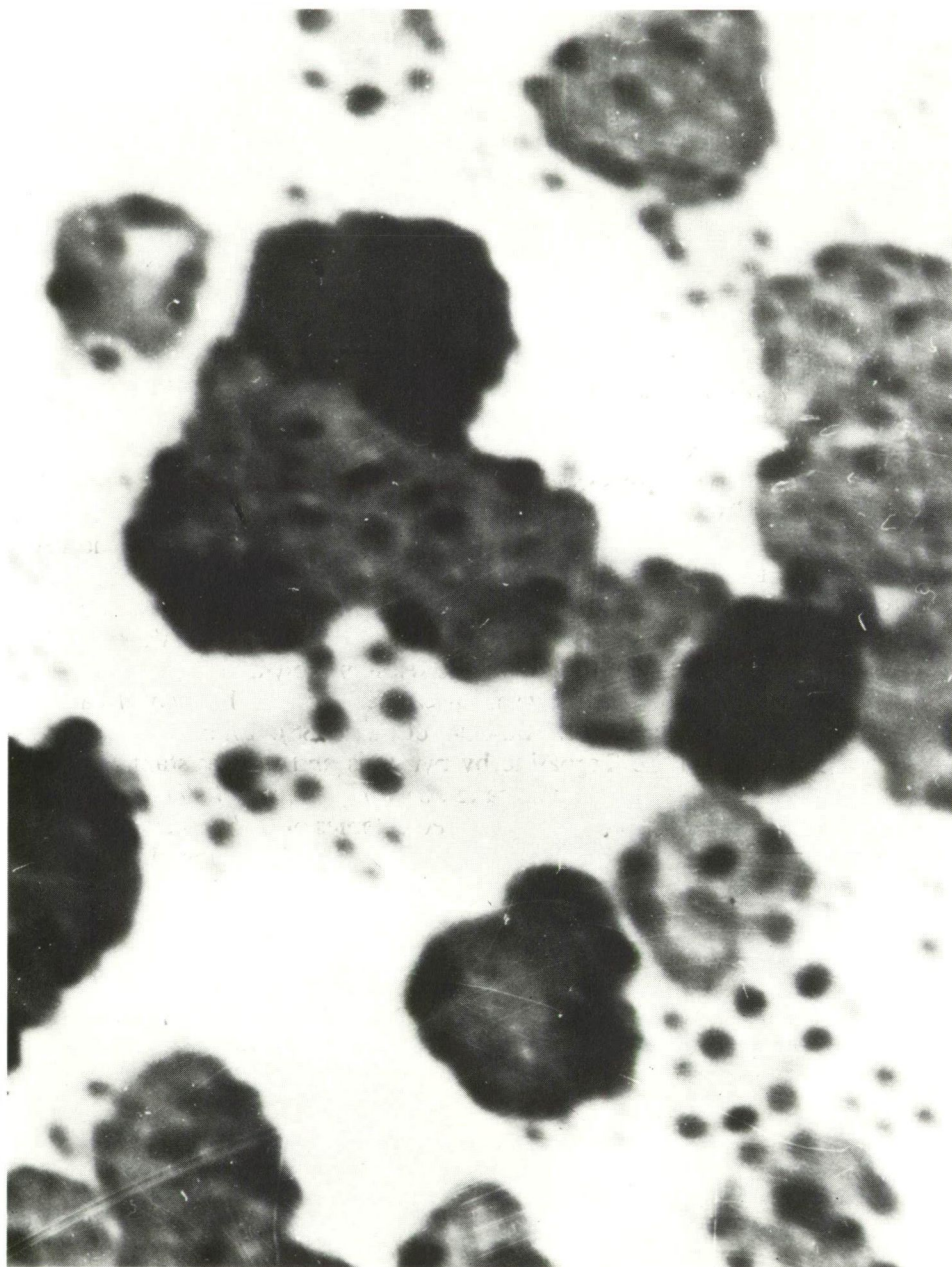


Plate 3.1.

TEM picture of the highly organized globular biopolymer units from the wall of *Botryococcus braunii* KÜTZ. The electron dense smaller globular units are well shown in larger ones. Experiment No 925 (10 mg. air dried material + 1 ml merkpto—ethanol, temperature 30 °C, length of time 48^{hrs}). Negative No 599. Magnification 3000.000 x.

further recent and fossil biological objects. It is clear that the investigation of the biopolymer system of the fossil material is much more complicated than those of the recent ones. That's why in the present day state of our research program of our investigations are mostly focussed to the Recent taxa. But taking into consideration our previous results were obtained by the combined methods on the *Botryococcus braunii* KÜTZ, from the oil shale of Pula, a new series of experiments were projected and is under elaboration. The concepts are as follows.

1. Use the so-called classical solvent and oxidizing method (2-aminoethanol, KMnO_4 aq dil., etc.)

2. Merkapto-ethanol as single solvent for relatively long times, maximum 15 days. After these processes, the remnants were fragmented, and investigated with the TEM method, with the instrument of Tesla BS—500.

Among the first results as a preliminary report the peculiar globular units were represented on the Plate 3.1. It seems to be interesting in our point of view. The highly organized structures are extremely resembling to the globular scheme on p. 63, 1.3. and 1.7., KEDVES (1989). Such globular units were observed at the first time on fossil objects.

This work was supported by the grant OTKA—2, 24/88.

References

- BERKALOFF, C., CASADEVALL, C., LARGEAU, C., METZGER, P., PERACCA, S. and VIRLET, J. (1983): The resistant polymer of the hydrocarbon-rich alga *Botryococcus braunii*. — *Phytochemistry* 22, 389—397.
- KADOURI, A., DERENNE, S., LARGEAU, C., CASADEVALL, E. and BERKALOFF, C. (1988): Resistant biopolymer in the outer walls of *Botryococcus braunii*, B race. — *Phytochemistry* 27, 551—557.
- KEDVES, M. (1983): Étude paléobotanique sur les schistes pétrolifères du Tertiaire supérieur de Hongrie. — *Rev. de Micropaléontologie* 26, 48—53.
- KEDVES, M. (1986a): Dégradation expérimentale des colonies du genre *Botryococcus* des schistes pétrolifères du Tertiaire supérieur de Hongrie. — *Acta Biol. Szeged.* 32, 39—48.
- KEDVES, M. (1986b): Komplex (LM, TEM és vékonyréteg kromatográfiás) vizsgálatok olajpala növényi mikrofossziliáin. (A complex study of plant microfossils of oil shale by LM, TEM and thin layer chromatography). — *Bot. Közlem.* 73, 25—32.
- KEDVES, M. (1989): Quasi-crystalloid biopolymer structures of the sporoderm and its highly organized degrees. — *Acta Biol. Szeged.* 35, 59—70.
- KEDVES, M. and ROJIK, I. (1989): Investigation of the biopolymer organization of partially degraded exines with the fragmentation method. — *Acta Biol. Szeged.* 35; 71—80.
- LARGEAU, C., DERENNE, S., CASADEVALL, E., KADOURI, A. and SELLIER, N. (1986): Pyrolysis of immature Torbanite and of the resistant biopolymer (PRB A) isolated from extant alga *Botryococcus braunii*. Mechanism of formation and structure of Torbanite. — *Org. Geochem.* 10, 1023—1032.
- MARTIN, H.A. (1973): The Palynology of some Tertiary Pleistocene deposits, Lachlan River Valley, New South Wales. — *Austr. Journ. Bot., Suppl. ser. 6*, 1—57.
- MAZANCOVÁ, M. (1960): Palynologický výzkum jilu v Okoli Ejpovic (Plzenská pánev). — *Cas. pro. miner. a geol.* 5, 265—274.

- MILDENHALL, D.C. (1977): Preliminary palynological thoughts on the Lower Miocene, Kawaran River, Central Otago. — Geol. Soc. (N. Z. Conf. 22nd, Queenstown, Program and Abstracts), 44.
- RAO, N.S.R. and MISRA, S.S. (1949): An oil-bearing alga from the Palana Lignite (?Eocene) of Rajputana. — Curr. Sci. 18, 380—381.
- SAH, S.C.D. and KAR, R.K. (1970): Palynological interpretations of paleoenvironments with reference to India. — Palaeobotanist, 19, 86—94.
- TRAVERSE, A. (1955): Pollen analysis of the Brandon lignite of Vermont. — Bur. Min. Repts. Invest. 5151, 1—107.
- WODEHOUSE, R.P. (1933): The oil shale of the Eocene Green River Formation. — Bull. Torr. Club 60, 479—524.

4. BIOPOLYMER ORGANIZATION OF PARTIALLY DEGRADED EXINES OF SACCATE GYMNOSPERM POLLEN GRAINS

Short communication

M. KEDVES₁, Á. PÁRDUTZ₂ and A. VÉR₃

1,3. Cell Biological and Evolutionary Micropaleontological Laboratory of the Department of Botany of the J.A. University, H-6701, P.O. Box 657, Szeged Hungary. 2. Institute of Biophysics, Biological Research Center of the Hungarian Academy of Sciences, H-6701, P.O. Box 521, Szeged, Hungary.

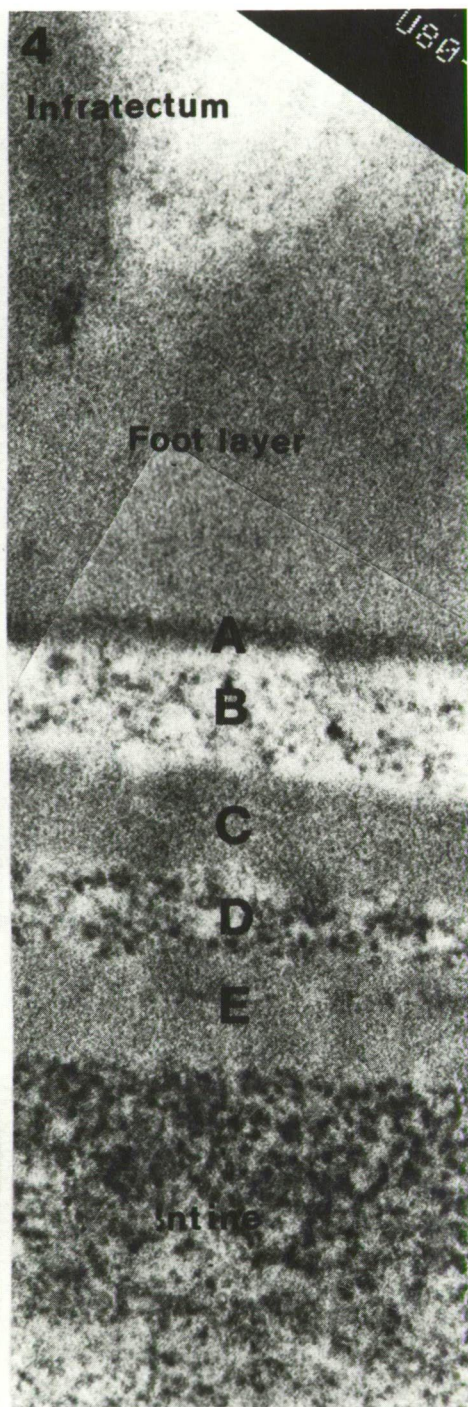
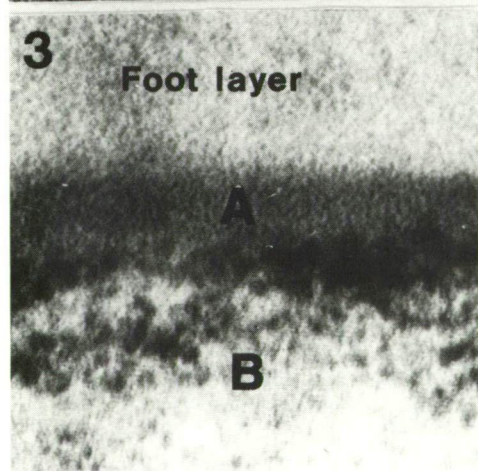
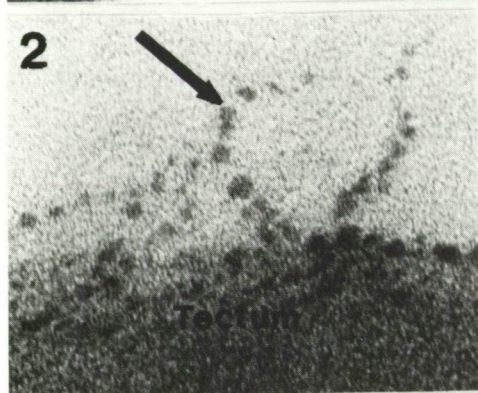
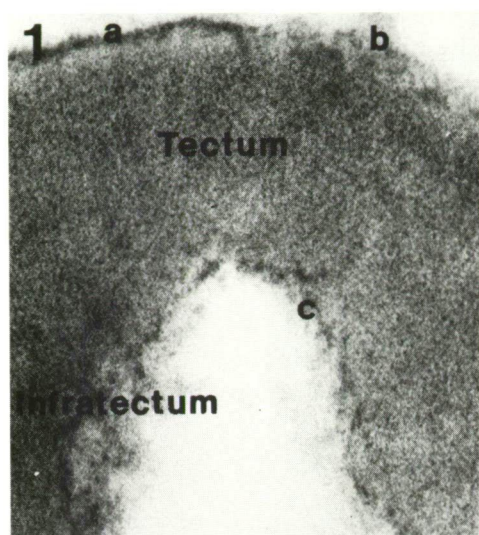
In a previous paper (KEDVES, 1988) quasi-crystalloid biopolymer lattice was described from the partially degraded exine of pollen grains of *Pinus griffithii* McCLELL. Using the modified Markham rotation method several methodical results were published or are in print (e.g.: KEDVES 1989, 1990, KEDVES and ROJIK, 1989, KEDVES et al. 1989, etc.). In the course of modelling the basic biopolymer unit of the quasi-crystalloid skeleton (GÉVAY and KEDVES, 1989) an idea emerged as follows. In all probability organic solvents of pentagonal molecular symmetry may be useful to dissolve the quasi-crystalloid skeleton. An opportunity to get direct informations about the molecular structure of the stabilizing components of the

Plate 4.1. ►

1. *Picea glauca* (MÓNCH.) VOSS.
Biopolymer organization of the partially degraded ectexine. Experiment No 680. Negative No 531. Magnification 250.000 x.
- 2, 3. *Pinus mugo* TURRA
2. Detail from the partially degraded tectum. Well shown is the characteristic glycogen molecular structure, marked with an arrow. Experiment No 634. Negative No 399. Magnification 500.000 x.
3. Biopolymer organization of the inner part of the ectexine and the further inner layers. A = Thin basal layer of the ectexine with strong electron affinity. B = Probably the intine. Experiment No 634, Negative No 393. Magnification 500.000 x.
4. *Picea glauca* (MÓNCH.) VOSS.
Detail from the partially degraded exine. A = This basal layer of the ectexine with strong electron affinity. B—E = Probably the lamellar endexine. The biopolymer organization of layers C and E is identical with those of the outer part of the ectexine. Layers B and D are similar to the intine on the basis of its biopolymer structure after degradation. Experiment No 680. Negative No 529. Magnification 250.000 x.

Experiment No 634: 20 mg. air dried pollen material + 5 ml tetrahydrofuran. Temperature: +5—6 °C, length of time: 12 days.

Experiment No 680: 20 mg air dried pollen material + 5 ml diethylether. Temperature: +5—6 °C, length of time: 25 days.



plant cell wall, which are interbedded in the frustrations (sensu NELSON, 1986) of the quasi-crystalloid lattice. The following solvents were used: n-pentane, tetrahydrofuran, pyrrolidine. Moreover diethylether was also employed. (Cf. SOUTHWORTH, 1974). Among the first results in the case of exines of *Picea excelsa*, *Pinus mugo*, *Pinus nigra* and *Pinus griffithii* we emphasize the following.

1. In general on the surfaces there is a thin protective layer with strong electron affinity (Plate 4.1., fig. 1a). After the disappearance of this layer the ectexine gets strongly damaged (Plate 4.1., fig. 1b, c).
2. Filaments of 5–6 Å corresponding to the glycogen molecular structure (network of chains, cf. DARNELL et al., 1986) were observed (Pl. 4.1., fig. 2.).
3. At the border line between ectexine and intine or further inner layers (Plate 4.1., 4A) can be distinguished by its stronger electron affinity only. The further differences in the ultrastructure are not the same (cf. Plate 4.1., fig. 3, 4).
4. In the centre of negative pentagonal basic biopolymer units of the exine small granular elements can be observed.
5. The negative quasi-crystalloid lattice seems to be same at the borders of the foot layer and the endexine.
6. In the ectexine highly organized biopolymer units were also observed (Plate 4.1., fig. 4).
7. Different solvents have different effects.

This work was supported by the grant OTKA—2, 24/88.

References

- DARNELL, J. LODISH, H. and BALTIMORE, D. (1986): Molecular Cell Biology. — Scientific American Books, Inc., New York.
- GÉVAY, G. and KEDVES, M. (1989): A structural model of the sporopollenin based on dodecahedrane units. — Acta Biol. Szeged. 35, 53–57.
- KEDVES, M. (1988): Quasi-crystalloid basic molecular structure of the sporoderm. — 7 Internat. Palynol. Congr. Brisbane, Abstracts, 82.
- KEDVES, M. (1989): Méthode d'étude des biopolymères de la paroi pollinique à structure quasi-cristalloïde. — Rev. de Micropaléontologie 32, 226–234.
- KEDVES, M. (1990): Quasi-crystalloid basic molecular structure of the sporoderm. — Rev. Palaeobot. Palynol. 64, 181–186.
- KEDVES, M. and ROJIK, I. (1989): Investigation of the biopolymer organization of partially degraded exines with the fragmentation method. — Acta Biol. Szeged. 35, 71–80.
- KEDVES, M. TÓTH, A., FARKAS, E., BELLON, A. and SCHMÉL, Á. (1989): Methodical problems of the biopolymer organization of partially degraded ectexine. — An. Univ. Budapestinensis de R. E. Nom. Geol.
- NELSON, D.R. (1986): Quasicrystals. Scientific American 254, 42–51.
- SOUTHWORTH, D. (1974): Solubility of pollen exines. — Amer. J. Bot. 61, 36–44.

5. BASIC ESTABLISHMENTS OF THE BIOLOGICAL OBJECTS MOLECULAR STRUCTURE CONTAINING QUASI-CRYSTALLOID SKELETON

Short communication

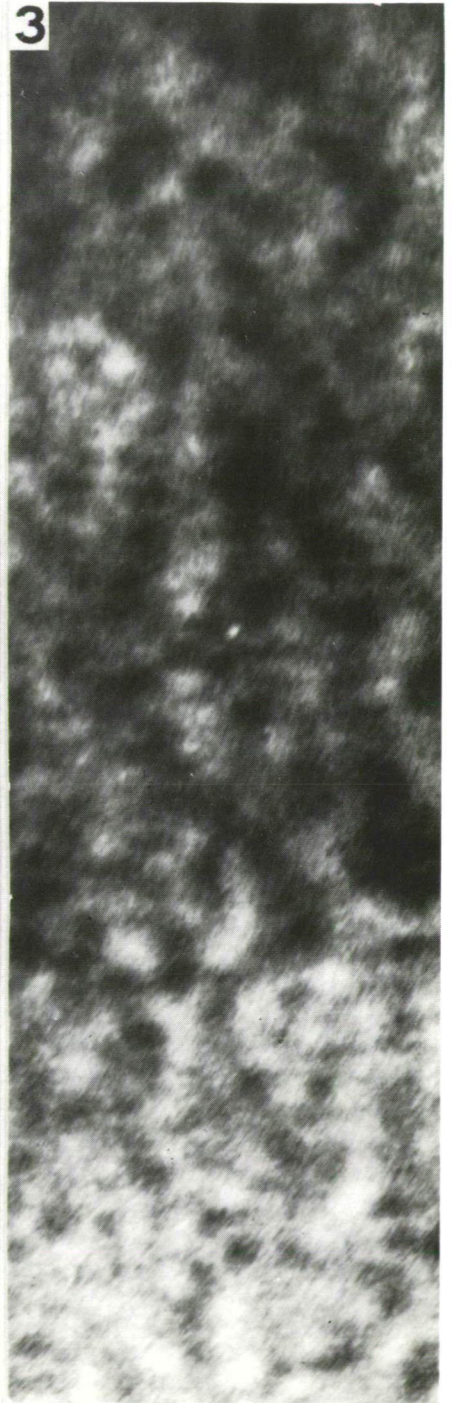
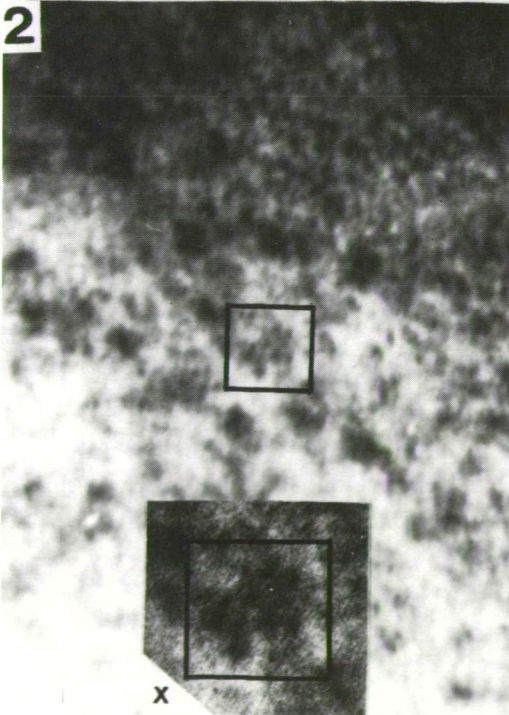
M. KEDVES₁, Á. PÁRDUTZ₂, E. FARKAS₃ and A. VÉR₄

1,3.4. Cell Biological and Evolutionary Micropaleontological Laboratory of the Department of Botany of the J.A. University, H-6701, P.O. Box 657, Szeged, Hungary, 4. Institute of Biophysics, Biological Research Center of the Hungarian Academy of Sciences, H-6701, P.O. Box. 521, Szeged, Hungary.

During our first observation of biopolymer structure of fossil angiosperm exines (KEDVES et al. 1974) globular units — size from 12 Å to 29 Å — were observed as units of the sporopollenin. Further smaller globular structures of 2–3 Å (p. 429) in diameter were also observed. Diameter of these units is below the resolution capacity of the TEM instrument (TESLA, BS-500). Later several experiments were made in order to establish the biopolymer organization of plant cell wall. The most important discovery was the quasi-crystalloid skeleton in the sporoderm (KEDVES, 1988). By the way, several new results of the modified Markham rotation method were published or are under publication.

We have the opportunity to take picture of 400.000 x (resolution 2–3.5 Å) with the new TEM instrument of the Biological Research Center of the Hungarian Academy of Science (OPTON 902). The previous short communication. (4.) touched — among others — the glycolipid molecular chains on the surface. We tried to prepare enlargements of 5 million of the above mentioned negatives, and on the basis of the first attempts we established as follows.

1. These high magnificated TEM pictures (Plate 5.1., fig. 2, 3.) of partially degraded plant cell wall gave more information about the fine molecular structure that weren't available on the pictures of the magnification of 1 million (Plate 5.1., fig. 1.).
2. There is an opportunity to observe the reflection of C-atoms in the organic molecular structure too (Plate 5.1., fig. 2.). All these units are below the resolution power of the new instrument.
3. Taking into consideration that the quasicrystals were discovered in rapidly cooled MnAl Alloy in consequence of the anomaly of the electron diffraction pattern (BURSILL and PENG JU LIN, 1985, SACHDEV and NELSON, 1985, AUDIER and



GUYOT, 1986, NELSON, 1986) it is the time to declare as a methodical postulatatum that:

In the case of quasi-crystalloid biopolymer skeleton contained biological objects the resolution power of the TEM instrument is irregular.

For this reason we have such molecular units or contours of the atoms in the organic molecules on the TEM negatives. The quasi-crystalloid character of the biopolymer structure creates this anomaly; to be more exact this extremely high resolution power.

Preparation of a synthetic three-dimensional molecular model on the basis of these new data is under preparation.

This work was supported by the grant OTKA—2, 24/88.

References

- AUDIER, M. and GUYOT, P. (1986): Al_4Mn quasicrystal atomic structure, diffraction data and Penrose tiling. — *Phil. Mag. Letters* 53, L43—L51.
- BURSILL, L.A. and PENG JU LIN (1985): Penrose tiling observed in a quasi-crystal. — *Nature* 316, 50—51.
- KEDVES, M. (1988): Quasi-crystalloid basic molecular structure of the sporoderm. — 7 Internat. Palynol. Congr. Brisbane, Abstracts, 82.
- KEDVES, M., STANLEY, E.A. and ROJIK, I. (1974): Observations nouvelles sur l'ectexine des pollens fossiles des Angiospermes de l'Eocène inférieur. — *Pollen et Spores* 26, 425—437.
- NELSON, D.R. (1986): Quasicrystals. — *Scientific American* 254, 42—51.
- SACHDEV, S. and NELSON, D.R. (1985): Order in metallic glasses and icosahedral crystals. — *Physical Rev. B*, 32, 4592—4606.

◀ Plate 5.1.

1. *Pinus mugo* TURRA
Biopolymer organization of the partially degraded tectum. Experiment No 634. Negative No 399. Magnification 1 million.
- 2, 3. *Pinus griffithii* McCLELL
2. Biopolymer structure of the partially degraded exine at the border of the endexine/intine. Experiment No 681. Negative No 401. Magnification 1 million, respectively (x) 2 million.
3. High magnified (5 million) picture of the ectexine/endexine or intine border. Experiment No 669. Negative No 435. Illustrated are the light and dark globular units and its linear and helical arrangement with ramifications.

Experiment No 634: 20 mg. air dried pollen material +5 ml tetrahydrofuran. Temperature: +5—6 °C, length of time: 12 days.

Experiment No 669: 20 mg air dried pollen material +5 ml pyrrolidine. Temperature: +5—5 °C, length of time: 25 days. Experiment No 681: 20 mg air dried pollen material +5 ml diethylether. Temperature: +5—6 °C, length of time: 25 days.

Chronicle

In order to commemorate the memory of Late Padma Shri (Mrs) SAVITRI SAHNI, the "Savitri Sahni Smarak Lecture Series" was organized on the occasion of her birthday on the 19th September, 1990 at Banquet Hall, Hotel Clarks Avadh, Lucknow at 4.30 PM. The inaugural ceremony and the programme was organized by dr. SHYAM C. SRIVASTAVA Secretary of the Birbal-Savitri Sahni Foundation, with the assistance of Mrs MADHU SRIVASTAVA.

His Excellency B. SATYANARAYAN REDDY, Rajyapal Uttar Pradesh, has very graciously consented to be the Chief Guest.

Programme

4.30 PM Reception

Vandana

Welcome to the Chief Guest by Secretary

Address by His Excellency B. SATYANARAYAN REDDY,
Governor of Uttar Pradesh

Address by Chairperson

Thanks by the Associate Secretary, Birbal Savitri

Sahni Foundation

5.15 PM Coffee

5.30 PM Lecture I: Dr. B.S. VENKATACHALA, Director, Birbal Sahni Institute of Palaeobotany, Lucknow.

Title: "The Past of the Green World".

6.00 PM Lecture II: Prof. M. KEDVES, J.A. University, Hungary

Title: "Aspects and Prospects in Palaeobotany".

6.30 PM Lecture III: Dr. JOHN RIGBY, Queensland Geological Survey, Australia.

Title: „Glossopteris an Enigma”.

In connection with this we publish the following.

Excerpts

from the "Will" of Late (Mrs.) SAVITRI SAHNI (1902—1985)

"It is my desire that in taken of my love and devotion to that fine and great spirit of my noble husband Late Professor BIRBAL SAHNI and in the pride of my illustrious husband's unique lofty ideals, a portion of my Ashes be strewn in the flower-beds around his "Samadhi" at the Birbal Sahni Institute of Palaeobotany, Lucknow.

To my Palaeobotanist colleagues the world over whom I hold in very high regard and affection, I offer my deep gratitude for their cooperation in the cause of science and towards fulfilment of our ideal, which has been a source of great encouragement to me. I express for them my unbounding affection and my all blessings follow them ever. I realise what great it is in life to have been given the great gift of receiving such unstinted affection from all over the Palaeobotanical world and I am beholden to them".



Photograph 1

His Excellency B. SATYANARAN REDDY Governor of Uttar Pradesh during his address.
Lecturers sitting in the front row (from left to right): DR. B.S. VENKATACHALA Director, DR. JOHN RIGBY and Prof. M. KEDVES.



Photograph 2

DR. B.S. VENKATACHALA Director, Prof. M. KEDVES and DR. SHYAM C. SRIVASTAVA Secretary of the Birbal-Savitri Foundation at the presidential desk.
Photographs from the courtesy of DR. SHYAM C. SRIVASTAVA.

With such noble feelings, the Late Padamshri (Mrs.) SAVITRI SAHNI founded Birbal-Savitri Sahni Foundation with the following programmes:

1. International Research Collaborative Programmes for exchange of Scientists between the Birbal Sahni Institute of Palaeobotany, Lucknow and Palaeobotanical organization abroad; would be known as "Birbal-Savitri Sahni Collaborative Research Programme".
2. Birbal-Savitri International Fellowships would be awarded to young Palaeobotanists/Earth Scientists for carrying out research in any specialised branch of palaeobotany in India.
3. Birbal-Savitri Sahni International Awards would be given alternative years to an outstanding scientist excelling in Palaeobotanical and allied fields which would carry cash prize of Rupees 25,000/— and a plaque in gold-silver. Nominations for such award may be forwarded to the Foundation between 10th and 26th April every year.
4. "Savitri Sahni Samman" founded by friends and admirers of Late (Mrs.) SAVITRI SAHNI for her dedication to the cause of Palaeobotany, would carry cash prize of Rupees 10,000/— including a medal which is to be given annually on 22nd January to a Palaeobotanist for outstanding research work.
5. "Savitri Sahni Smarak Lecture" instituted with cost donations from well wishers of Late (Mrs.) SAVITRI SAHNI carries token honorarium of Rs. 5,000/— for an invited lecture every year on 19th September in any specialised field of Palaeobotany. The Lecture would be published as monograph under the auspices of the Birbal-Savitri Sahni Foundation. These programmes are meant to promote palaeobotanical and allied sciences the world over, for which Late (Mrs.) SAVITRI SAHNI donated every bit of her belongings to the Nation and entrusted to Birbal-Savitri Sahni Foundation. The entire residence of SAHNI's situated at the banks of river Gompti is planned to be converted into Museum-cum-Guest House and a "Palaeograden" is also being planned to develop at the site where her last remains were consigned to flames.



B 135180

Responsible for publication: M. Kedves
 Responsible editor: Gy. Györfy
 Cover by I. Biró—Halász
 Set in New Times 10/11 point
 Szegedi Nyomda, Szeged